RANGE: Undergraduate Research Journal (2023)

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An Introduction - Range: Undergraduate Research Journal

Annie Fukushima

2023 marked an important year for undergraduate research at the University of Utah. The Office of Undergraduate Research led campus-wide efforts to host a state-wide conference on undergraduate research through the [Utah Conference on Undergraduate Research](). Over 600 students from 10 different universities in Utah presented their research at the University of Utah, representing a diversity of fields. Slogans proliferated social media assets pushed out through the Undergraduate Studies campaign, "inquisitive minds welcome," "inquiring minds welcome," and even, "what if we brought the best undergraduate research in the state. We did!" Concluded research that was presented at UCUR, maybe found sprinkled throughout this issue. Additionally, reflected here in the 148 contributions, are the 140 students who graduated this 2023 spring with an [Undergraduate Research Scholar Designation,]() a scholarly designation that appears in student transcripts after

conducting a minimum of two semesters of research, presenting their research, and submitting their research to this journal.

The dynamic research reflected in this issue has many forms: articles, abstracts, and research reflections. And the subjects covered here, cuts across the disciplines, representing the vital role that higher education has as a public good. Higher education, and educational environments that foster experiences and opportunities for students to participate in the research enterprise leads to not only students as ambassadors of our institutions. It also means, when they graduate they take with them the skills, curiosities, and important questions that will support them in their post-graduation endeavors.

I want to commend our students who conducted research this 2022 – 2023 academic year. While the US is emerging from global pandemic, the fatigue, the mental health impact, the real social, political, and economical realities impacting our communities, also impact students. Student researchers are the embodiment of persistence, where they materialized their research as publications, presented their work, worked in community, and beyond with research endeavors spanning the creative, scientific, humanistic, social behavioral, and clinical. The challenges that continue to impact our society, were the inspiration for many of these projects, where mirrored in these contributions are resolve to solve today's problems and answer societal questions through rigorous disciplinary and transdisciplinary research.

Therefore, this issue of *RANGE: Undergraduate Research* is an homage to the dynamic research occurring in the state of Utah. It is an invitation to inquiring minds to enter this 2023 issue.

To close, I end with gratitude to our contributors, research mentors (faculty and graduate student alike), Eliana Massey

(undergraduate research leader 2022 – 2023), Eli Covarrubias, Cindy Greaves, Jude Ruelas, Shelly Parker, and Megan Shannahan, and OUR program partners, who have provided important support and leadership to undergraduate research that materialized in this issue of *RANGE: Undergraduate Research Journal* (2023), Volume 24, Issue 1, formerly known as the *Undergraduate Research Journal.*

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SECTION I

David Eccles School of Business

1. Employee Voice in Saudi Arabia

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Faculty Mentor: Dr. Amelia Stillwell (Management, University of Utah)

Abstract

Within organizations, employees are faced with the decision when they have a new idea or suggestion: either speaking up (i.e. voice; Morrison, 2011), or remaining silent. Employee voice has been shown to increase creativity in organizations. Yet, the research around employee voice has been largely focused on Western workplace contexts. Little research has examined voice in other cultural contexts, even though culture is known to guide norms around discussion and dissent. In this study, we look deeper into how employees voice in the Saudi Arabian work context.

Introduction

Employee voice– the voluntary decision to speak up with suggestions to improve the organization (Morrison, 2011)– has important positive impacts for organizations. As a form of idea sharing, voice also increases creativity (Carnevale, J.B., Huang, L., Crede, M., Harms, P., and Uhl-Bien, M. (2017). Employee Voice also increases organizational performance through the transfer of knowledge (Argote & Ingram, 2000). Therefore understanding and enabling voice is vital for an organization's success.

Research on employee voice has mainly focused on Western workplaces, implicitly assuming that these findings generalize to other cultural contexts, such as Saudi Arabia. Yet, organizations don't exist in isolation from their broader cultural context: they are influenced by the national culture in which they are embedded (Kwon, Farndale, 2016). Saudi Arabia is a high power distance culture with tight norms and high context, meaning that in Saudi culture, hierarchy is well-established and socially accepted (i.e. high power distance; Hofstede, 1982), norms are strict, and there is low tolerance for deviance (i.e. tightness; Gelfand et al., 2011), and social context shapes the meaning of interpersonal communication (i.e. high context; Hall, 1959, 1976). The combination of these norms likely creates a different context for voice than that observed in Western cultures, where voice has previously been studied. Thus, existing research on employee voice may not generalize to Saudi Arabia, as culture influences norms for appropriate behavior in a given context (Lapinski & Rimal, 2005; Zhou et al., 2015). Indeed, cultural tightness and hierarchy, in particular, influence tolerance of dissent, a closely related construct to voice (Blair & Bligh, 2018; Harrington & Gelfand, 2014; Morgan & Kelly, 2021). Because research suggests that both power distance and cultural tightness are associated with a lower

tolerance for dissent and deviance (Blair & Bligh, 2018; Harrington & Gelfand, 2014), we argue that these differences in Saudi culture shape workplace voice norms in ways not understood by previous research.

To address this missed opportunity, we sought to better understand the strategies employees use to voice in Saudi Arabia – a hierarchical, tight, and high context work context. Saudi Arabia's culture is high in power distance, cultural tightness, and context. As a high power distance culture, in Saudia Arabia, "less powerful members of an organization accept and expect power to be distributed unequally"(Hofstede,1982), meaning that hierarchy is mutually accepted and valued within society. Saudi Arabia is also culturally tight, meaning there is low tolerance for the violation of social norms (Gelfand et al., 2011; Harrington & Gelfand, 2014). In addition, Saudi Arabia is high context, meaning that individuals depend more on the social *context* of a message to guide its meaning, compared to low context cultures that put more weight on the *content* of the message (Hall, 1959, 1976). Individuals in high-context cultures are more sensitive to social rules, especially when addressing individuals of higher status in the social hierarchy. As such, initial research suggests that high-context-oriented individuals are less likely to voice in the workplace, but also that these behaviors depend greatly on the content of their message and their relationship with the listener (Ward, Ravlin, Klaas, Ployhart, & Buchan, 2016) Thus, multiple elements of culture in Saudi Arabia– high power distance, tight social norms, and high context– likely suppress the traditional forms of research studies in management research.

Though Saudi Arabian cultural norms around hierarchy, tightness, and context likely discourage dissent as it has

traditionally been studied, the high context of Saudi culture may offer novel avenues for employees to indirectly voice contrary opinions. We expect that despite the cultural pressures against voice and dissent in Saudi workplaces, the high context culture may offer heretofore unrecognized means for employees to voice their opinions in a culturally appropriate way. For example, employees may suggest alternatives as a way to dissent from the current course of action or utilize analogies and metaphors to express their opinion without being confrontational. To investigate this possibility, we leverage narrative analysis of real workplace experiences from employees in Saudi Arabia to examine how norms around the voice in Saudi culture influence workers' choices and strategies to voice at work. Using these narratives as well as existing measures of cultural values, we explore how lower-level employees in Saudi Arabia navigate age-based hierarchy to put forward their ideas and express concerns.

We will contribute to the practice and theory on voice in organizations in three key ways. First, we generate new and important insights on how to engage in effective workplace communication in a heretofore under-researched cultural context: Saudi Arabia. This knowledge comes at a particularly important time in Saudi Arabia's development, The nation's relatively recent moves to open its national economy to foreign capital and organizations necessitate a greater understanding of how to engage in culturally appropriate business communication in this context. Second, we suggest that existing measures of voice might be improved by attention to additional strategies employees use in highly hierarchical, tight, and high-context work environments. Given the reliance of the field on voice measures developed in Western contexts, the present work suggests these measures likely neglect the

unique forms employee voice may take in hierarchical, tight, and high context cultures in general and in Saudi Arabia in particular. Finally, we expand the field's understanding of what constitutes voice behavior at work, arguing that cultural context may facilitate more indirect forms of voice in certain cultures. While we focus here on Saudi Arabia, the tools and strategies utilized by employees in this particular cultural context may also be effective in other cultures characterized by high power distance, tight norms, and/or high context. Taken together, these contributions offer a more culturally-comprehensive understanding of workplace voice behavior.

Methodology

The aim of this study was to find how employees in the Saudi Arabia cultural context voice their opinions at work. In order to reach the maximum number of participants while maintaining their anonymity, we developed an online survey that participants could take anywhere. Given that existing measures related to voice were developed and validated in Western cultural contexts, we acknowledge that some of the existing measures we utilize in this research may miss valuable insights on voice in the Saudi context. Thus, our survey included both existing survey measures of culture and voice and open-ended qualitative responses.

Procedure

We distributed the survey through King Saud University in Saudi Arabia. Though we targeted students and student workers via this method of distribution, anyone over the age of 18 qualified to participate. We focus our analysis on participants who indicated they had prior work experience. We also aim to distribute the survey to small and medium businesses to their employees. Participants will be presented

with a consent form, and then they will proceed to answer demographic questions, such as age, gender, etc. After demographic questions, they proceed to answer qualitative questions in which they will need to type their answers either in Arabic or English. The next set of questions is a scales question in which they will need to indicate to which degree they agree or disagree with a given situation that they will be asked about. Participants were able to choose and toggle between viewing the survey in English or Arabic.

Measures

Translation Procedure

The most challenging aspect of this research was the translation of research materials to Arabic. We faced several obstacles in translating some of the questions to fit the Saudi cultural context. Cultural differences embedded in how Arabic and English are used led to challenging decisions about word choice. For example, for the item "it is important for him/her that no one should shame [them]" (Schwartz, 1992), we wrestled with finding words that would convey shame in the way intende(*Innovation Strategy, Voice Practices, Employee Voice Participation, and Organizational Innovation | Voice & Dissent Across Cultures | Zotero*, n.d.)d in the question because in Arabic words have different meanings based on the context in which they are stated. Another challenge came from the very basic assumptions underlying the certain cultural value questions. For example, one question asked how much participants agreed that "It is important to [them] that the weak and vulnerable in society be protected' (Schwartz, 1992). In Saudi Arabia, it's culturally believed that the weak and vulnerable should be protected, specifically by the government. Therefore the question was not capturing valuable information. We decided to keep the question to be consistent with the scale. In order

to overcome these obstacles, we consulted two native experts outside of the research team– a Saudi professor of linguistics and a professional Saudi social science researcher– in order to better determine the proper terms to convey the meaning of questions in the local Arabic dialect used in Saudi Arabia.

Qualitative Items

After filling out demographic information, participants answered a series of detailed, open-response qualitative questions. Participants responded to the following:

For the first question, we were aiming to capture an instance when employees either voiced their opinions or withhold them; this will enable us to capture valuable insight into how employees deal with moments of disagreement or voice. "Think of a situation in the workplace that you experienced when you had a different opinion from someone who was of higher status than you (A manager or a senior manager in the organization)". The second question aimed at digging deeper to understand if the age hierarchy played a role in interaction " Was this person older, younger, or same age?" In the third question, we aimed at understanding if age played a difference in their decision to voice."What was the gender of this person?" In this question our aim was to understand if the decision to voice was affected by other people witnessing the conversation."Did others witness the conversation, or was it a private conversation?" In this question, we were trying to harness more information about the interaction that took place "Describe the context and what happened in those moments." in this question we were wanted to understand if there were any consequences from the persons managers or leaders "What stopped you or motivated you to speak up?" Finally, in this question what was the reaction of other other employees,

specifically followers "What happened after you spoke up? Did others acknowledge your disapproval?"

Survey Items

Schwartz's Cultural Values. We utilized the Schwartz values model in a survey form. Participants were asked to think of a person, and that person was subjected to different value propositions. Participants then had to indicate the degree to which that person was similar to them. For instance, one item stated: "It is important for him to form his views independently;" participants rated each item on a 6-item Likert scale (1 = Not like me at all – 6 = Very much like me).

Cultural Tightness-Looseness. We utilized Gelfand's model in a survey form. Participants were asked a series of questions regarding social norms and situations and asked the degree to which they agreed or disagreed to the given statement. For example, "There are many social norms that people are supposed to abide by in this country." to which participants answered each item on a 6-item Likert scale (1 = Strongly Disagree – 6 = -strongly agree).

Belief in the co-production of leadership. In this survey, we were trying to understand how participants viewed the role of followers in relation to their leaders.. Participants had to indicate how much they agreed to each statement. For example, one item stated: "Followers should be on the lookout for suggestions they can offer to superiors." participants answered each item on a 6-item Likert scale (1 = Strongly Disagree – 6 =strongly agree).

Constructive resistance. In this survey, we aimed to understand what tactics participants utilized when their manager to leader asked them to finish a task they thought was not useful.

Participants were presented with a series of tactics; they had

to indicate the degree in which they utilized that particular tactic. For example, "I explain that I think it should be done a different way." participants rated each item on a 6-item Likert scale (1 = I cannot remember ever using this tactic – 6 = I almost always use this tactic)

Current Progress and Discussion

In this study, we sought to better understand the voice strategies used by employees in Saudi Arabia, an underresearched cultural setting characterized by hierarchical, tight, and high- context cultural norms. We translated the survey and submitted it to King Saudi University in Saudi Arabia for approval and distribution. Though we have obtained initial approval, we are still waiting on the final approval to publish the survey. Once we have data, we anticipate several themes are likely to appear in workers' narratives; specifically, we expect workers to observe that maintaining vs. risking relationships, the age-based hierarchy between individuals, and individual manager influence, are each important factors determining when and how they choose to voice at work.

Relationships. In Saudi Arabia, the relationship between the follower and the leader is likely significant in determining an employee's choice to voice. In this context, relationships are significant to one's social status, and relationships outside the workplace permeate the organization's boundaries. Thus, the interpersonal relationship between leader and follower, connecting leader and follower outside of the organization, may carry more weight in Saudi Arabia than in Western cultures.

Age. Age and power hierarchies play an important role in setting expectations for followers and leaders in Saudi Arabia; thus, it may be difficult for employees to voice opinions

because of the norms to listen and value the opinions and directions of the elderly in Saudi culture, even if they share a good relationship. In Saudi culture, respect for the elderly is highly valued and regarded. For example, when an older person makes a decision, it is best to trust in them due to their age and experience. As such, it may be hard for younger employees to voice their opinion due to age differences.

Manager Influence. Managers generally set normative expectations for employees in the workplace and thus may amplify or dampen the cultural norms that govern these relationships. For example, a manager might endorse tighter social norms and convey these expectations to employees; as a result, employees might perceive that voice and dissent come with greater interpersonal risk. Therefore, the manager's own cultural values may influence how employees choose to voice.

References

Argote, L., & Ingram, P. (2000). Knowledge transfer: A basis for competitive advantage in firms. Organizational behavior and human decision processes, 82(1), 150-169.

Blair, B. A., & Bligh, M. C. (2018). Looking for leadership in all the wrong places: The impact of culture on proactive followership and follower dissent. Journal of Social Issues, 74(1), 129– 143.

Carnevale, J. B., Huang, L., Crede, M., Harms, P. D., & Uhl-Bien, M. (2017). Leading to engage: A motivational model of leadership, employee engagement, and perceived effectiveness. Journal of Organizational Behavior, 38(3), 439-458.

Gelfand, M. J., Raver, J. L., Nishii, L., Leslie, L. M., Lun, J., Lim, B. C., ... & Yamaguchi, S. (2011). Differences Between Tight

and Loose Cultures: A 33-Nation Study. Science, 332(6033), 1100-1104. DOI: 10.1126/science.1197754

Hall, E. T. (1959). The Silent Language. Doubleday.

Hall, E. T. (1976). Beyond Culture. Anchor Press/Doubleday.

Harrington, J. R., & Gelfand, M. J. (2014). Tightness–looseness across the 50 United States. PNAS Proceedings of the National Academy of Sciences of the United States of America, 111(22), 7990–7995.

Hofstede, G. (1982). Dimensions of national cultures in fifty countries and three regions. International Journal of Psychology, 17(1-4), 41-49.

Kwon, B., Farndale, E., & Park, J. G. (2016). Employee voice and work engagement: Macro, meso, and micro-level drivers of convergence? Human Resource Management Review, 26(4), 327–337.

Lapinski, M. K., & Rimal, R. N. (2005). An Explication of Social Norms. Communication Theory, 15(2), 127–147.

Morgan, J., & Kelly, N. J. (2021). Inequality, exclusion, and tolerance for political dissent in Latin America. Comparative Political Studies, 54(11), 2019-2051.

Morrison, E. W. (2011). Employee Voice Behavior: Integration and Directions for Future Research. The Academy of Management Annals, 5(1), 373–412.

Schwartz, S. H. (1992). Universals in the content and structure of values: Theoretical advances and empirical tests in 20 countries. In M. P. Zanna (Ed.), Advances in experimental social psychology, Vol. 25, pp. 1–65. Academic Press.

Schwartz, S. H., Vecchione, M., Fischer, R., Ramos, A., Demirutku, K., Dirilen-Gumus, O., Cieciuch, J., Davidov, E., Beierlein, C., Verkasalo, M., Lönnqvist, J.-E., & Konty, M. (2012). Refining the Theory of Basic Individual Values. Journal of Personality and Social Psychology, 103(4), 663–688.

Yuan, F., & Zhou, J. (2015). Effects of cultural power distance on group creativity and individual group member creativity. Journal of Organizational Behavior, 36(7), 990-1007. doi: 10.1002/job.2022.

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2. Research Reflection by Rajeh Albugami

Rajeh Albugami

Faculty Mentor: Dr. Amelia Stillwell (Management, University of Utah)

This experience has changed the way I think about research and research papers. Doing research and having to go through and edit and re-edit has enabled me to understand better the comprehensive process that researchers go through to produce a concise, well-written paper. The guidance of my mentor has been essential in the success of my undergraduate research. I enjoyed doing through this experience, and it has opened my eyes to the world of research and the importance and impact of it. After this experience, I'm motivated and encouraged to do more research.

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3. Art-Secured Lending and Evaluating the Loan-to-Value Ratio in Art and Real Estate Lending Markets

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Faculty Mentor: Jeffrey Coles (Finance, University of Utah)

A Senior Honors Thesis
Date of Submission: April 21, 2023

Abstract

I analyze the lending terms for loans with art as collateral. The standard loan-to-value (LTV) ratio offered by private banks is 50% of the value of a piece/collection, while for other tangible

assets, such as real estate, LTV is often 80%. I use a linear regression model with common U.S. stock indices as my independent variables to compare systemic and idiosyncratic risk for art and real estate. My analysis indicates that differences in these risk characteristics explain in part the substantially lower LTV ratio for art versus real estate. I also examine concerns about market liquidity, ownership, and authenticity of art as they pertain to LTV.

1 – Introduction

Fine art is a complex market that attracts some of the wealthiest individuals in the world. The most coveted painters regularly sell for tens to hundreds of millions of dollars depending on the appetite of a small group of collectors. It is a market that is opaque, loosely regulated, and inaccessible to the average individual. Art also has an increasingly active lending market, where collectors can take out loans with their art collections serving the function of collateral. By this I mean that the art serves as security for the repayment of the loan. This is similar to how one can borrow against their house in promise of repayment.

I compare the art market with the U.S. real estate market under the expectation that risk characteristics of art returns, and how those characteristics differ from those of housing returns, influence the terms of art-secured loans. Additionally, I discuss why private banks, who are creating some of these loans, find value in the market for loans with art as the collateral.

A reason that fine art is an attractive asset to collectors is the fact that art is aesthetically and spiritually valuable. It can often embody the

personal values of collectors and represent their own aesthetic sensibilities. Individuals and families end up developing long-lasting relationships with paintings, in which the presence of a piece represents an aspect of their legacy. Aside from the aesthetic dividend art pays (Etro & Stepanova, 2021, p. 108), collectors are drawn to the status of owning museum-quality works of art in their homes since they can show off their collections to their social and professional networks. There is also data to suggest that making fine art a component of one's portfolio can be valuable in terms of both portfolio diversification (Mei & Moses, 2002, Table 1) and potential appreciation in value of the art. For these and possibly other reasons "Ultra-High Net Worth Individuals" (UHNWI) are interested in art collecting.

Art tends to have a less-active and less-liquid market than more typical securities, such as a debt or equities. To capitalize on the economic value of an artwork, collectors traditionally had to find a private-buyer or auction the work, where values exist only as estimates and sales can take months from start to finish. The low reliability of pre-auction estimates (Yu & Gastwirth, 2010, p. 850) and the time to facilitate sales make it challenging for collectors to use their art to cover short-term cash needs. Art lending, which was first formally offered as a service by Citigroup's Private Bank in 1979 (Neuhaus, 2015, pp. 146-147), has been rapidly

expanding to allow collectors to use their art as a security for a loan.

An asset-secured loan is a type of loan where the guarantee of repayment is backed by another asset. Loans backed by real estate, such as mortgages, are asset-secured loans. In like manner, art is a tangible object with value as collateral. The lending party, typically a bank, will lend at a fraction of the value of the collateral called the loan-to-value ratio (LTV). This ratio is important because it represents the risk banks are willing take in the event the loan isn't repaid. Failure of repayment, also known as a default, allows the lending party to repossess the underlying security of an asset-secured loan. The LTV is then effectively how much the lending party paid in order to acquire the collateral. The LTV ratio for fine art, across the industry, tends not to exceed 50% (Medelyan, 2014, p. 652). Alternatively, real estate typically has a LTV of 80% (Lack, 2016, p. 47), which is higher than fine art.

Many UHNWIs have used their art collections to finance art-secured loans (Medelyan, 2014, pp. 651-653). According to John Arena (2022), director of Deutsche Bank's art lending team, the art-secured lending market was expected to reach $31.3 billion in 2022, an 11% growth rate from the previous year. This, however, represents a fraction of the total value within the art market. According to Deloitte, the total value of UHNWI art and collectibles was estimated to be $1.49 trillion in

2020 (Arena, 2022). Art and other collectibles on average comprise 9% of high-net worth individuals' portfolios (Li et al., 2022, p. 2). Currently, there still appears to be a large addressable market. The double-digit growth rates of recent years could be potentially sustainable for the foreseeable future.

   Using data that tracks the returns of art and real estate markets, I examine the extent to which the risk characteristics between these two asset classes offers explanatory power for the differences between LTV ratios. With this data, I conducted a regression analysis using common U.S. stock indices as the independent variable. The regression models estimate both the systemic and idiosyncratic risk of market returns for real estate and art. Differences in these components of risk suggest why LTV ratios are different for these two asset classes and why banks are likely to provide more value on a home-equity loan versus a loan against a fine art collection. I find that art tends to be less sensitive to market returns than real estate (meaning that art has lower systemic risk) and that real estate tends to have slightly higher idiosyncratic risk. The latter is due to greater volatility in past real estate returns and lower exposure to general economic conditions. The statistical differences in these risk components supports the argument that LTV ratios of 50% are justified despite surface level similarities between these two asset classes.

2 –Lending Collateral: Real Estate as an Analogy for Art

To understand why art-secured loans have a lower LTV ratio relative to other real assets, I choose real estate as an analogy. Real estate provides a strong point of comparison to fine art for several reasons. Not only do both have active lending markets, but they are also tangible and provide some kind of service flow. For instance, real estate can be lived in or rented to others and art provides the collector with status and aesthetic pleasure. They also share the characteristic that the underlying asset is truly unique. Art, even produced by the same artist, can differ significantly in value based on size, condition, and historical importance (Sotheby's). In like manner, even two houses that are structurally identical will be on different plots of land, have different maintenance concerns, and consequently will be valued differently. Additionally, fine art and real estate both share liquidity concerns where the sale of the asset will typically take months to find the right buyer and include a seller's fees.

Nevertheless, there are several key areas where these assets differ, which could potentially create differences in their respective lending markets. Homeowners are present in many economic demographics, whereas the art that qualifies for art-lending is mostly owned by the highest echelon of wealthy individuals, the UHWNIs. The

service flow that real estate generates provides a more tangible source of value since it tends to be easier to quantify cashflows from rent than the value of the consumption stream from proximity to fine art. There are also many risks that are unique to art, such as higher risks of art going unsold at auction, authenticity of individual paintings, and lack of a transparent and fully accurate system to record titles of ownership. These risks would be considered idiosyncratic risks of art since they are particular to art as an asset class.

The similarities and differences between real estate and art provide me with a starting point for attempting to explain the LTV discrepancy between these assets, 50% in art versus 80% in real estate. Since these two assets share a few core similarities, the discrepancy in LTV ratios might be explained by art having risk characteristics that differ from those of real estate. A bank can hedge market risk but, unlike real estate, the art market is relatively thin and the idiosyncratic risk of the value of art as collateral is not easily diversified. The difference in LTV ratios potentially would be explained by the idiosyncratic risk of art being greater than that of real estate. Nevertheless, I tested both systemic and idiosyncratic risk to explore whether we might gain further explanatory power into why banks aren't

providing the same loan value for art as they do with real estate.

3– Landscape of the Art Lending Market

3.1 Role of Private Banks

There are several institutions that are willing to lend against art as collateral, such as luxury pawnshops, auction houses, and private banks (Neuhaus, 2015, pp. 146-149). Each serves a specific niche in the lending market, but private banks are particularly interesting because it is not directly obvious how a relatively small business within private wealth management would be capable of art lending as compared to a large investment bank. Such banks have traditionally supplied capital via more typical securities. On the other hand, many private banks rely on third parties to engage in art-lending, rarely repossess artwork, and do not charge high enough interest rates to generate significant revenue. These are characteristics of services that tend to be atypical for investment banks. These few considerations pose the question of why this service exists in its current form.

There are two primary factors that help explain why private banks are willing to take a risk on fine art. First, is that this service cross-fertilizes other businesses within the private banks by creating a strong relationship with clients that might lead to business elsewhere within the bank. Since the trades of an individual client are confidential, it is challenging to estimate the value that this service provides for a bank. Nevertheless, a 2018 survey from Deloitte and ArtTactic Ltd states that 40% of private banks are looking to make art-lending a strategic focus in the coming year ("Cash in on

your Picasso", 2019). This is consistent with the strong growth this service has seen in recent years, and why it is projected to expand further. Second, a lower LTV for fine art compared with other assets, such as real estate, might suggest that the private bank is taking on less risk. But if art is riskier collateral, banks will loan less relative to the underlying value of the collateral until the risk of such collateral is tolerable to the bank. A lower LTV for fine art suggests that private banks see art as a riskier asset than real estate. On the other hand, inconsistent with the assertion that art is riskier, literature suggests that art market index returns are steadily 1% above inflation (Zhukova et al., 2020, p. 9). The counterargument is that perhaps such art-market indices do not fully capture the risks associated with liquidity, authenticity, and ownership.

3.2 Loan Terms

Art-secured loans provided by the private banks are typically structured as a revolving line of credit (Blackman, 2015), which is a form of debt that allows those who are receiving the loan to draw upon the credit as required. There is a limit placed on the credit based on the value of the collateral relative to the LTV. One of the benefits of a revolving line of credit is that it allows for more flexibility since withdrawals and payments can be made at any point within the maturity of the loan. Maturities are generally around two-years, and it is

uncommon for them to be longer than five years (Medelyan, 2014, p. 652). The most similar loan for real estate is a second-lien mortgage, which is a more junior loan secured by the same house as a more senior facility. These loans, typically referred to as home-equity line of credits (HELOC) can be structured as a revolving line of credit or a term loan.

Private banks also have requirements for the art they will accept as collateral. First, the art must be appraised annually and authenticated by a third party (Ray, 2015, p.18), typically an independent authenticator. For the bank to establish a security interest, a lien, on the collateral, due diligence is required by Article 9 of the Uniform Commercial Code (Medelyan, 2014, pp. 645-646). Single works of art are typically insufficient to comprise collateral. Instead, usually a collection of works would be collateralized. JPMorgan Chase's private bank requires that the collection be diversified, a minimum of five pieces be put up as a security interest, and the value of each piece must exceed at least $750,000 ("Case in point", 2016). Other banks require that the art in question must be valued at least $10m (Blackman, 2015). Having at least five pieces that meet these conditions implies that, to qualify for a loan, the client of the bank likely has a fairly substantial art collection already. This parallels how the bank will typically lend against a portfolio of stocks, as opposed to a single stock,

in order to reduce idiosyncratic risk. Similarly, in the case of art, this might be strategy to reduce the risk around particular pieces in the collection which might produce competing ownership or authenticity claims in the unforeseeable future. To further mitigate risk, private bank's structure these loans as recourse loans (Neuhaus, 2015, p. 146), which allows them to repossess other assets in their client's portfolio in the rare case of a default. Since wealth managers have unique insight into the myriad of different investments that their client owns, finding alternative sources to repay the defaulted debt would likely not be a challenging barrier, although it could potentially damage a client relationship.

Interest rates on art-secured loans tend to be low ranging from 0.71% to 3.25%, approximately the range of 30-year U.S. treasury notes, which is considered a relatively risk-free asset (St. Louis Federal Reserve, 2023). On the contrary, 30-year mortgage rates on real estate have typically been between 3% to 6% in the last two decades (Freddie Mac, 2023). In 2015, for instance, gaming magnate Steve Wynn borrowed against his art collection at an interest rate of only 1% ("Case in point", 2016). Offering low rates suggests that private banks, to some extent, view art lending primarily as a relationship-building business.

Numerous sources have found that a maximum 50% Loan-to-Value (LTV) ratio is standard among

private banks. According to Citigroup's private bank head of art advisory Suzanne Gyorgy, a LTV of 50% with a minimum of $10m tends to be the standard for their loans against art (Blackman, 2015). This sentiment was echoed by John Arena (2022), head of Deutsche Bank's private bank art advisory team. Simply put, that means a single painting valued at $10m would be able to produce a line of credit with a maximum of $5m. Real estate, however, most commonly has a LTV ratio of 80%, which implies that a home worth $10m would be able to secure a loan of $8m.

3.3 Advantages of Art Lending

What appears to be the primary advantage of art loans from the bank's perspective is the ability to distinguish themselves and drive deal activity through other businesses. The teams that facilitate these art loans typically fall under the umbrella of an art advisory team within private wealth management services. Due to the unique nature of alternative investments, such as fine art, having an experienced advisory team allows the bank to appeal to clients with substantial collections of art in their portfolios, with the expectation that these clients will take advantage of other wealth management services the bank has to offer. Additionally, investment banks look to their private wealth management clients to place securities or secure funding for upcoming deals (Weinberg, 2017). Offering art lending, especially at relatively lower interest rates, keeps their clients happy and thus generates more business for the banks.

Another advantage for the private banks comes from the fact that foreclosure is uncommon, and

the banks do not want to put their clients in a position where they take on more debt than they could pay back. John Arena (2022) of Deutsche Bank claims he has never seen a foreclosure happen, even with 27 years of being in the business. Considering that many collectors have art that has been passed down, or they themselves have felt an aesthetic connection with the work they purchased, repossessing a piece of art would likely significantly damage a client relationship. Banks are able to mitigate default risk through relatively low LTV ratios, in addition to the fact that they have extended insight into their client's portfolio. Arena also states that while a collector's main hesitation about using their art as collateral is the fact that they might lose it, the bank will rarely extend a loan to somebody who they believe cannot pay back the loan through some other source on their balance sheet.

From the perspective of collectors, there are several advantages for securing a loan with their art. According to the Deloitte/ArtTactic survey of collectors, over 50% of collectors said they would be interested in the service with 53% saying they would use the loan to acquire more art, 38% saying they would use the money to finance existing business activities, and 9% saying they would use it to refinance prior loans, possibly ones with higher interest rates than an art-secured loan (Blackman, 2015). Although the majority of collectors

interested in this service are looking to expand their art collections, there are many who intend to use this new source of capital to finance other areas of their portfolio, besides their collection. Another advantage of art lending, particularly in the United States and Canada, is the ability for collectors to keep their art in their homes (Neuhaus, 2015, p. 147). Considering the service flow of art, this means that collectors can continue to enjoy the aesthetic and social satisfaction that fine art provides. Additionally, private banks benefit from this rule since it implies that they will not be burdened with finding storage or covering insurance for the collateral, just conducting the due diligence required to confirm that storage and insurance exist.

The art lending business appears to be propelled by the belief that if you keep your clients happy by offering a unique service, then they will be more likely to do business with you elsewhere. Transitioning to a discussion of the quantifiable risks that banks take by extending loans on art, this belief supports why banks are willing to take on a seemingly risky venture, while charging low interest rates, which is typically inversely related to risk. Not every client of a private bank will have an art collection and even if they do, they still must qualify for the collateral restrictions that banks put in place. That implies that it is likely that this

service only comprises a small fraction of the investment bank's total businesses.

4 – Hypothesis

To test why an art-secured loan has a lower LTV ratio than real estate, I hypothesize that this was due to differences in risk characteristics between these two asset classes. Specifically, I suppose that the value of art is less sensitive to external market forces than real estate, a greater proportion of risk can be hedged with real estate than with art, and that there is greater noise (unhedgeable risk) for art than real estate. Such suggests the hypothesis that a reason that LTV ratios are lower for art than real estate is the fact that the risk characteristics of art are not only more difficult to hedge, but also more difficult to forecast.

The null hypothesis I test is that art is not less sensitive to external market forces than real estate, art has a greater proportion of risk that can be hedged than real estate, and that there is not greater noise within the models for art. If I accept the null hypothesis, I reject that risk characteristics are partially responsible for the discrepancies between the LTV ratios. This may imply that idiosyncratic forces such as liquidity, authenticity, and lack of ownership transparency are likely the primary drivers behind the discrepancies in LTV ratios.

Support for the hypothesis does not reject that idiosyncratic factors do not contribute to lower LTV ratios for art-secured loans, but instead offers further explanatory power that differences in

relative LTV ratios may also be driven by quantifiable market forces.

5 – Data

5. 1 Sources

I conducted my analysis using three popular U.S. stock indices as my independent variables. The indices I employ are generally accepted as approximately representative of U.S. stock market performance. The Russell 3000 represents the broadest view of the performance of U.S. publicly traded equities since it tracks the returns of the top 3000 U.S. publicly traded companies, ranked by market capitalization, essentially the capital size of the underlying company. The S&P500 gives insight into the top 500 U.S. publicly traded companies ranked also by market capitalization but adjusted to consider the volume of shares traded publicly. The final index is the NASDAQ which is calculated by all the equities which are traded on the NASDAQ stock exchange.

For my art dependent variable, I primarily relied on the art indices provided by Art Market Research (AMR), which is considered the industry standard for tracking the performance of a variety of collectible markets including art, wine, cars, and watches. I have also included data from the ArtPrice indices, which use a similar methodology as AMR, but produce somewhat different results for analogous categories. In regression models estimated using the ArtPrice indices, the coefficients on stock market returns were not statistically significant. Accordingly, for the purposes of my analysis I use the AMR indices. The AMR index methodology applies a repeat-sales

model from data taken from analysts at AMR, as well as from data independently provided by auctioneers. I choose two of their indices Art 100 and Contemporary Art 100 which compile auction results from the top 100 trading global and contemporary artists, respectively. Considering the $750,000 requirement that some banks place on art to qualify as collateral, this would likely be reflect the value of work of those 100 artists. Nonetheless, there is plenty of art that is valued above $750,000 that is not the work of the 100 artists. The ArtPrice indices I utilize are from ArtPrice Base 100, which also segments by the top 100 global and contemporary artists and also includes data given a distinct medium (painting, sculpture, drawing, etc.). I also include the ArtPrice Global index in all of my tables, as a means for comparison, although I base my conclusions on the AMR data.

For my real estate dependent variables, I choose the National Association of Real Estate Investment Trusts Residential (NAREIT) and the S&P/Case-Shiller U.S. National Home Price Index (Case-Shiller). The NAREIT is calculated via the net operating income of the underlying homes, which provides insight into the performance of the residential real estate market in the U.S. Case-Shiller uses a repeat-sales model to track systematic changes in home prices. Although Case-Shiller's methodology is most similar to the AMR, it had no significance to any of three U.S. equities indices,

which appears to run counter to the conventional expectation that the performance of real estate at least somewhat reflects the performance of the stock market. For this reason, in addition to the fact that the NAREIT was always statistically significant with the three stock indices, I choose to base my results on the NAREIT. Since any of the conclusions using the Case-Shiller index could also be explained by randomness in the model, I believe that NAREIT was a better source for my analysis.

5.2 Summary Statistics

Relevant to my analysis of the risk characteristics of fine art and real estate is the mean and standard deviation of the returns for their indices. Table 1 shows that the average returns I found for the global and contemporary art indices appear consistent with other research (Zhukova et al., 2020, p. 9) that demonstrate that annual art returns average about 1%, with contemporary markets being slightly higher. The NAREIT returns an average of about 2.9%, which is higher than all of the art indices.

The standard deviation, or volatility, of the AMR indices is lower than NAREIT. On the other hand, the volatility of the ArtPrice indices is greater than that of NAREIT. Relying on the AMR data, it is then even more confounding why an asset that is less volatile than real estate, is given a lower LTV ratio. A possible explanation for this is that private banks are not lending against an index of art as a

security, but rather individual paintings which may not reflect the performance of the index. Depending on the group of artists who created those pieces, there could be dramatically different risk profiles, even more nuanced than individual categories such as time-period or medium. While it is possible to analyze the returns of an individual's artworks, the infrequency on which a particular piece is traded means that very few data points will typically exist for a given artwork. On the other hand, investment banks have security vehicles such as Collateralized Debt Obligations which are designed to reduce the risk of default on any particular piece of real estate. This is likely one of the many complications private banks have when deciding on an appropriate risk tolerance for art.

6 – Methods

For my analysis of the risk characteristics of art versus real estate, I estimate a linear-regression model using quarterly return data over the time-period of January 1998 – December 2022. This provides 100 observations and 99 return observations that span over two decades. These decades include both strong stock market growth as well as the major financial crisis of 2008. This allows the regression to test the response of art and real estate to both positive and negative market pressure. I chose a regression model to analyze the discrepancies around the LTV of art

versus real estate because it yields estimates of the most fundamental risk statistics of art, including the sensitivity of the returns on the relevant asset (art or real estate) to stock market returns (β), the proportion of variation in art returns explained by the regression model (R2), and the proportion of variation unexplained by the model (1-R2). Table 1 includes the average returns and standard deviation of every index that I analyzed over the course of my research. In Tables 2-4 I include relevant regression statistics of the indices I chose to focus my analysis on relative to a single independent variable. In Table 5, I calculate the systematic risk and noise of the dependent variable for each independent variable. The calculation in Table 5 uses the standard deviation along with the percentage of variance within the regression model to produce calculations for systemic risk and noise which represent the level of hedgeable risk of the dependent variable (σ*R2), as well as the level of unhedgeable risk of the dependent variable (σ*(1-R2)). Charts 1 and 2 are a representation of the regression of Art 100 and NAREIT against the Russell 3000 as the independent variable. This was included to provide a visual summary of the differences between the relationship of art and real estate to the stock market over the last 100 quarters.

7 – Results

7.1 Sensitivity and Systemic Risk

This section outlines the results included in Tables 2-5. I first explain the results of the sensitivity (β) comparison between fine art and real estate and then compare my results for the level of systematic risk for the two assets.

The β of Art 100 (Table 2: β = -.24) tends to be slightly negatively sensitive to the Russell 3000, S&P500, and has almost no connection to the NASDAQ index. In Chart 1, this appears to be primarily due to a few outliers in the third quadrant. This is consistent with other researchers (Mei & Moses, 2002, Table 1) in its application to the present day that art does provide a diversification benefit in a well-diversified portfolio. The sensitivity of Contemporary Art 100 (Table 2: β = -.35) is similar, although its negative sensitivity is slightly amplified. The NAREIT (Table 2: β = .73), however, has strong positive sensitivity to the movements of the independent variables. This appears to suggest that housing prices are impacted by or associated with the performance of the stock market. This is borne out in my data. The results are consistent with the first part of my hypothesis, specifically that art has less sensitivity to the stock market than real estate.

This provides some evidence that there may be systemic risk characteristics of art that can explain why art-secured loans typically carry a lower LTV ratio than the loans secured via real estate. All of the regressions that I have included in my research demonstrate that the sensitivity of the dependent to the independent variable (β) tends to be higher for real estate than it is for art. It might be the case that since art does not appear to be as sensitive to the market as real estate, investment banks do not

see their macro-economic views of markets to be particularly useful in this business. Since it appears that their data is not a strategic advantage in forecasting the art market, let alone individual pieces, it is expected that they would hesitate in providing more loan to value than they would with real estate. Although art has been shown to be a relatively stable, low-returning asset, which is consistent with my analysis, it is not reactive to the stock market, which might make it challenging to find ways to reduce risk.

Art has significantly less hedgeable risk than real estate by a factor of about six for the Russell 3000 in Table 5 (Art100 = .005 & NAREIT = .028). In Table 2, the R2 statistic for the Art 100 (.08) is about three times smaller than the NAREIT (.3). This discrepancy becomes amplified to six when multiplying by the variance of returns since the standard deviation of the NAREIT (σ = .092) is almost double that of the Art 100 (σ =.058). The measurement of systemic risk for art (Art100 = .005, Contemporary Art 100 = .007) strongly implies that almost none of the risk of art can be hedged using stock market indices. The measurement of systemic risk for real estate (NAREIT = .028), albeit small, represents a much larger proportion of risk that can be hedged. Systemic risk is relevant to the LTV ratio of these assets since it is the amount of risk that the banks can diversify away when creating loans. This brings me to the second part of my

hypothesis, which is that a greater proportion of risk can be hedged with real estate than with art. The availability of derivative securities to reduce systemic risk justifies why banks are willing to lend at LTV ratios of 30% more for real estate than they are with art.

The result for systemic risk demonstrates that real estate has significantly more hedgeable risk than art. Hedgeable risk determines the proportion of the underlying asset's risk that can be reduced by the bank, thus banks would be more likely to provide a lower LTV for an asset that they cannot hedge their risk for. All three stock index tables support the hypothesis that the reason banks are willing to provide more value for the collateral of real estate instead of art is because they are better able to reduce the risk of real estate, by a factor of about 6. The risk of not being able to hedge art is partially offset by the consideration that private banks are taking a macro-view of their client's portfolio when deciding to extend an art-secured loan. Risk is also partially reduced by the collateral requirement of a minimum of five pieces. Finally, since banks are only lending 50% of the value of the artwork, they can reduce their overall exposure and avoid having to deal with the complications of hedging that this analysis demonstrates.

7.2 Idiosyncratic Risk

Included in my analysis of the risk characteristics of fine art versus real estate are

their statistics for noise, also considered the measurement of idiosyncratic risk. Table 5 demonstrates that art has a slightly smaller statistic for noise than the NAREIT (Art 100 = .053, NAREIT = .064). There is still some support for the third part of my hypothesis, however, considering that Art 100 has a greater 1- R2 than the NAREIT (Table 2: Art 100 = .92, NAREIT = .7). This observation is grounded by the comparison of Chart 1 (Art 100 – Russell 3000) and Chart 2 (NAREIT – Russell 3000) since the points on Chart 2 follow a clear upward trend versus the seemingly random distribution of data points in Chart 1. The result that art has a slightly smaller statistic for noise was in part caused by the standard deviation of art being smaller than real estate (Art 100 σ =.058, NAREIT σ = .092). In summary, although the returns on art tend to be less volatile than real estate, there is significantly more unexplained variance when regressing art and real estate against a major U.S. stock index. This is consistent with the third component of my hypothesis, despite the fact that there is slightly more unhedgeable risk for real estate. This is due to the returns on real estate being more volatile, rather than because my regression model was better able to explain the variance. When controlling for standard deviation, the movements of these popular stock indices do not explain the

distribution of points for art as much as they do for real estate.

Considering that the function of a bank is generally to avoid positions where risk is poorly understood and difficult to hedge, it follows that they would use LTV ratios as a lever to reduce their overall risk exposure to art. While they could charge higher interest rates to reflect the greater risk of art, this runs counter to the philosophy of this service being one of relationship-building. Instead, lowering LTV ratios allows borrowers to receive substantial loans on their art in addition to improving the ability of those clients to pay back the principal without creating unnecessary risk for the bank.

The larger proportion of unexplained variance in the regressions using art indices might be explained by three primary risk factors that are unique to art as an asset class: liquidity, authenticity, and ownership. Traditionally, banks are not in the business of facilitating art-related transactions, providing authenticity opinions, and tracking ownership of art. These roles are typically specialized third-party services that require expertise. Alone, these factors can significantly impact the value of a given artwork, and thus deserve their consideration in attempting to explain why art has more unexplained variance than real estate.

7.2.1 Liquidity

Liquidity represents how fast an artwork can be sold for acquisition or appraised value. The sale of an artwork can often take months, insofar as the right type of auction may not be immediately available (Li et al., 2022, p. 2). Every piece of art has a unique value, even those that are essentially the same artwork such as a print. Coupled with the fact that the market for art is so thin, with relatively few buyers and sellers, as compared to real estate, what an estimator sets for the value of art may not accurately forecast the sale price (Yu & Gastwirth, 2010, p. 850). An auction sale for an artwork will typically have a reserve price, which must be met or exceeded for the transaction to be facilitated. In many cases that reserve price is not met or no bids are made, which leads to around 40% of auctioned items going unsold (Bruno et al., 2018, p. 833-834). An item failing to achieve a sale at auction indicates that the demand for that piece was not as strong as it was originally thought to be. This event often leads to the artwork being reappraised at a consistently lower value (Ashenfelter & Graddy, 2011, Figure 2) or being shelved for a future auction date when market demand is believed to be significantly different.

7.2.2 Authenticity

The authenticity risk of art also presents challenges that can significantly affect the value of an individual artwork. Authenticity refers to whether the artwork can be attributed to the artist whose name is associated with the work. Art from established, blue-chip, artists is a prime victim of counterfeiting. Successful forgeries can be worth millions of dollars. Advances in machine learning for the application of art authenticity opinions

likely would improve the process of conclusively determining authenticity (Łydżba-Kopczyńska & Szwabiński, 2022, pp. 17-18) yet it is still estimated that 40-50% of contemporary art that is circulating in the market is inauthentic (Li et al., 2022, p. 2), although verifying this estimation is challenging. Due to the legal liability of issuing a formal opinion on whether a piece of art is authentic, many authentication experts are hesitant about giving conclusive opinions. Even the slightest expression of doubt can significantly devalue a work. Additionally, there are no legal qualifications around being considered an art expert, and due to the opaque nature of the art market, it is challenging to establish whether an authentication expert has a financial interest in the authenticity of a particular artwork (Bandle, 2015, p. 382). These factors represent a relevant risk for banks, as authenticity is one of the main drivers of value for a blue-chip artwork. Paintings that are found inauthentic would be worth only a fraction of what the value of an authentic painting would be. Additionally, there is a contagion effect on the value of any painting of an artist whose work is forged as soon as the media reports of even rumors of a forgery on another work (Li et al., 2022, p. 13). By requiring multiple pieces for a loan to be established, banks can mitigate some of this risk, but it is another reason why they prefer loans to be paid back instead of repossessing the collateral.

7.2.3 Ownership

Private banks must navigate ownership risk when dealing with fine art. In the U.S. real estate market, the vast majority of all transactions are recorded in the Public Recorder of Deeds office (Pearson, 2015, paras. 12-13). The information on the Public Recorder of Deeds is public, so it is not challenging to determine whether an individual actually has a valid title of ownership in the case of real estate. Art, on the other hand, has no such public ownership record, most of the market is private, and for the auction houses, which tends to be the most public market for art, very little information is available about auctioned works. Although the blockchain has offered new opportunities for authenticating ownership digitally (Fairfield, 2022) and companies such as Verisart have been issuing digital certificates of authenticity, there is still no widely accepted public practice resembling the Public Recorder of Deeds. Traditionally, the closest analog in the art market for recording deeds are catalogue raisonnés, but they can be inaccurate and fragmentary, sometimes even including forged works. The existence of ownership records such as certification and reference in art literature, however, does have a positive effect on price (Li et al., 2022, p. 23). The absence of accurate ownership records implies that it is possible for a private bank to establish a security interest in an artwork that their client

doesn't legally own. Since the client never possessed legal ownership, a repossession is not able to produce a superior title and the bank could essentially lose ownership of the collateral. Although it is possible to reduce this risk through ownership insurance, it is still a unique risk of art that banks must consider when offering art-secured loans.

8 – Additional Considerations
8.1 Review of Analysis

This analysis provides a starting point for attempting to explain the discrepancies in LTV ratios using data from art and real estate indices. In this section I will outline some of the strengths and weaknesses of my approach, as well as some areas for further research on this topic.

An advantage of using a simple regression model to compare the risk characteristics of art and real estate is that it allowed me to compare statistics that tend to be more general in conclusion than more specific data analysis techniques. In the absence of information about the actual credit-scoring model of private banks for art-secured lending, these broader statistics allowed me to formulate a hypothesis that might explain the discrepancies around LTV ratios for art and real estate secured loans. The sources I used were chosen because they are also some of the broadest indicators of performance for particular asset classes.

Due to the simplicity of the model, there are also some weaknesses that could be accounted for by including other data analysis methods or other sources. For instance, in every regression I ran I was comparing two variables. More specific research into the risk characteristics of the art market by segment might employ a multiple linear regression model to account more accurately for how different sectors of the art market are responding to the stock market. Additionally, the loans available for residential real estate purchased by UHNWI might be significantly different than a typical 30-year mortgage, which was the LTV ratio I compared. I also used mortgage terms that are provided by commercial banks who could potentially have a different risk tolerance for lending than private banks which are selling the art-secured loans. Nevertheless, I did not find any evidence that a LTV ratio of 80% would not apply to a private bank loan which allowed me to find it acceptable for the purpose of this research.

There were also several unresolved concerns when comparing the data between similar indices. The data from AMR and ArtPrice, which are two separate data companies tracking the same market, produce different results when regressed against the same independent variable. For instance, the β for Art 100 (AMR) in Table 2 is -.24 versus Global Art (ArtPrice) .26, almost opposites of each other. A possible explanation in the discrepancies

between AMR and ArtPrice is that auction house data tends to be proprietary and public announcements of sales, which could have found their way into either of these indices, are not typically adjusted to consider variables within the final sale price such as buyer/seller fees. The regression statistics for Case-Shiller were also troubling since it had almost no sensitivity to the market, which goes against the generally accepted belief that real estate prices are at least somewhat related to stock market performance. Also, the NAREIT and the Case-Shiller index had almost no sensitivity to each other. This is problematic because they are tracking the same underlying asset, despite using two different methodologies. An additional investigation into the discrepancies between these data sources, for both art and real estate, might be required to measure the risk characteristics more accurately.

For further research, I believe that many of the collateral requirements for art-secured loans would be worthwhile to explore. For instance, whether there is selection bias in the artwork that is collateralized, whether there are restrictions on the medium of the collateral, and whether artwork from certain artists is deemed too risky to be collateralized. This research also didn't consider the role of auction houses and luxury pawnshops, two other art-secured loan providers that serve a different customer demand than private banks.

8.2 Further Research

There is much research to be continued on this topic, and it is unclear how the value of art will continue to evolve in response to the growth of art-secured lending markets. Due to the confidentiality around what art is really being held as collateral, it is unclear how much status an artist must have for their artwork to be eligible for a loan. While there are industry standards, such as the LTV ratio of 50%, there are a lot of unknowns to the public about what makes a particular private bank's art loan unique. Furthermore, there is evidence of institutions using art loans to tap into their liquidity during difficult times (Medelyan, 2014, pp. 651-652). There is strong possibility that museums and other arts institutions will look at lending against their art, possibly the art that is storage as well, to cover costs, instead of laying off employees or reducing spending elsewhere. This could dramatically alter the way that these institutions think about their balance sheet and could influence the way that they choose to serve the public. This would require reconciling differences across various art indices in addition to finding real estate indices that more accurately represent the holdings of UHNWI's.

9 – Conclusion

Despite the risks of art as a collateral, private banks are still insistent on developing this service for their clients. Banks minimize risk by choosing

to lend at relatively lower LTV ratios and by doing their due diligence on their client's portfolios to ensure they have the capital to repay the loans. The risk-return characteristics of art compared with real estate justify lower LTV ratios. By comparing the performance of art and real estate indices with the performance of the stock market over the last 100 quarters, real estate has significantly higher sensitivity to performance in the stock market. Additionally, real estate has more hedgeable risk than art, allowing banks to reduce their overall exposure to real estate as an asset. While the data analysis demonstrated that real estate does have more noise than the art indices, I have determined that this is being driven by past volatility of real estate over art, not by the models having more explanatory power on the variance of real estate. These findings demonstrate that real estate is better understood by the market than art. Accordingly, private banks would be willing to take a bigger risk by providing higher LTV ratios with real estate than art.

  The primary idiosyncratic risks of art, which are liquidity, authenticity, and ownership, might explain the greater unexplained variance in the regressions ran against art. All of these significantly affect not only the value of art, but also the potential complications around trying to turn art into cash. The lengthy time periods to sell art would mean that banks would be holding art

on their balance sheet, without the guarantee that they will be able to get the 50% of the appraised value that they lent. Since authenticity is one of the primary drivers of a blue-chip artwork's value, a work of art even rumored to be inauthentic could significantly impact the value of the collateral. Telling a forgery from an authentic work is a complicated and expensive task, despite not getting a guarantee that the work of art is authentic. In the case of a piece of art being improperly owned, banks run the risk of losing the painting in a costly legal battle. Even if there are no competing ownership claims at the time of creating the loan, there is no guarantee that during the life of the loan claims won't be made. These idiosyncratic factors are other risks that banks must consider when expanding their service for loans against art.

Based on the available research, it appears that a private bank's goal in making art loans is not that their clients will default, and they will be able to repossess the underlying collateral. Additionally, it appears that banks are not using interest rates to generate additional revenue. Investment banks are not in the business of trading art, although their executives may be, and repossession or high interest rates would go against the central philosophy of this service, to improve client experience. Repossession for private banks is extremely rare, almost unheard of, and private

banks minimize this risk by ensuring that clients have liquidity in other areas of their portfolio to pay back the loan in the event of default. Additionally, due to the myriad of risks explained above, having art on the balance sheet further complicates risk management for investment banks. Lending against art as collateral is something that can be analyzed due to similar asset characteristics with real estate, but it is unlikely that banks will look at this service as a strategy to improve their revenues directly. Instead, private wealth managers will continue to advertise this service to their UHNWI clients with substantial art portfolios to encourage them to use the private bank for all their financial management needs and indirectly create value in other divisions of the investment bank.

Tables

Table 1: Summary Statistics

Table 1: Summary Statistics

Index	Mean	St. Deviation
Art 100	1.57%	0.06
Contemporary Art 100	2.08%	0.08
Global Art	1.08%	0.14
Contemporary Art	2.47%	0.14
Post-War Art	1.61%	0.08
Painting	0.23%	0.04
Sculpture	0.12%	0.05
Photography	0.84%	0.09
Drawing	0.66%	0.08
Print	1.28%	0.07
NAREIT	2.92%	0.09
Case-Shiller	1.28%	0.02
S&P500	1.63%	0.07
Russell 3000	1.69%	0.07
NASDAQ	2.50%	0.11

Art100 is an Art Market Research index of the top 100 global artists ranked by annual sales. Contemporary Art 100 is an Art Market Research index of the top 100 contemporary artists ranked by annual sales. Global Art is an ArtPrice index of the top 100 global artists ranked by annual sales. Contemporary Art is an ArtPrice index of the top 100 contemporary artists ranked by annual sales. Post-War Art is an is an ArtPrice index of the top 100 post-war artists ranked by annual sales. Painting to Print are ArtPrice indices of the top 100 artists of a particular medium ranked by annual sales. NAREIT (National Association of Real Estate Investment Trusts Residential) is an index of the net operating income of residential real estate in the U.S. Case-Shiller (S&P/Case-Shiller U.S. National Home Price) is an index of home-prices using a repeat-sales model. S&P500 is an index

of the top 500 U.S. publicly traded companies. Russell 3000 is an index of the top 3000 U.S. publicly traded companies. NASDAQ is an index of all the U.S. publicly traded companies which are traded on the NASDAQ exchange.

Table 2: Russell 3000 Regression Statistics

Table 2: Russell 3000 Regression Statistics					
	α	β	R^2	$1-R^2$	P-Value
ART100	0.02	-0.24	0.08	0.92	0.004
Contemporary Art 100	0.03	-0.35	0.09	0.91	0.002
Global Art (Artprice)	0.01	0.26	0.02	0.98	0.184
NAREIT	0.02	0.73	0.30	0.70	0.000
Case-Shiller	0.01	0.05	0.03	0.97	0.098

To analyze the effect of the Russell 3000 on art and real estate, I use a linear regression model. α indicates the intercept coefficient of the regression line. β indicates the sensitivity of the dependent variable to the Russell 3000. R2 indicates the proportion of the variance for the dependent variable explained by the Russell 3000. P-value (.05) is the statistical significance values < (.05) are statistically significant and values > (.05) are not statistically significant.

Table 3: NASDAQ Regression Statistics

Table 3: NASDAQ Regression Statistics					
	α	β	R^2	$1-R^2$	P-Value
ART100	0.02	-0.10	0.03	0.97	0.078
Contemporary Art 100	0.02	-0.16	0.04	0.96	0.040
Global Art (Artprice)	0.01	0.08	0.00	1.00	0.533
NAREIT	0.02	0.32	0.13	0.87	0.000
Case-Shiller	0.01	0.02	0.01	0.99	0.461

To analyze the effect of the NASDAQ on art and real estate, I use a linear regression model. α indicates the intercept coefficient of the regression

line. β indicates the sensitivity of the dependent variable to the NASDAQ. R2 indicates the proportion of the variance for the dependent variable explained by the NASDAQ. P-value (.05) is the statistical significance values < (.05) are statistically significant and values > (.05) are not statistically significant.

Table 4: S&P500 Regression Statistics

Table 4: S&P500 Regression Statistics					
	α	β	R^2	$1-R^2$	P-Value
ART100	0.02	-0.23	0.07	0.93	0.008
Contemporary Art 100	0.03	-0.35	0.08	0.92	0.004
Global Art (Artprice)	0.01	0.25	0.02	0.98	0.218
NAREIT	0.02	0.75	0.30	0.70	0.000
Case-Shiller	0.01	0.06	0.03	0.97	0.072

To analyze the effect of the S&P500 on art and real estate, I use a linear regression model. α indicates the intercept coefficient of the regression line. β indicates the sensitivity of the dependent variable to the S&P500. R2 indicates the proportion of the variance for the dependent variable explained by the S&P500. P-value (.05) is the statistical significance values < (.05) are statistically significant and values > (.05) are not statistically significant.

Table 5: Risk Characteristics

Table 5: Risk Characteristics

	Systemic Risk	Noise
Russell 3000		
ART100	0.005	0.053
Contemporary Art 100	0.007	0.073
Global Art (Artprice)	0.002	0.134
NAREIT	0.028	0.064
Case-Shiller	0.001	0.022
NASDAQ		
ART100	0.002	0.056
Contemporary Art 100	0.003	0.077
Global Art (Artprice)	0.001	0.136
NAREIT	0.012	0.080
Case-Shiller	0.000	0.022
S&P500		
ART100	0.004	0.054
Contemporary Art 100	0.007	0.074
Global Art (Artprice)	0.002	0.134
NAREIT	0.027	0.065
Case-Shiller	0.001	0.022

To compare the risk characteristics between art and real estate across the three independent variables, I calculated the systemic risk and noise using the standard deviation of the dependent variable and the R^2 of the regression, respectively. The calculation for systemic risk, which measures the proportion of diversifiable risk, is ($\sigma^* R^2$). The calculation for noise, which measures the proportion of risk that is not diversifiable is ($\sigma^*(1-R^2)$).

Charts

Art 100 - Russell 3000

y = -0.2375x + 0.0197
R² = 0.0825

NAREIT - Russell 3000

y = 0.7266x + 0.0169
R² = 0.3037
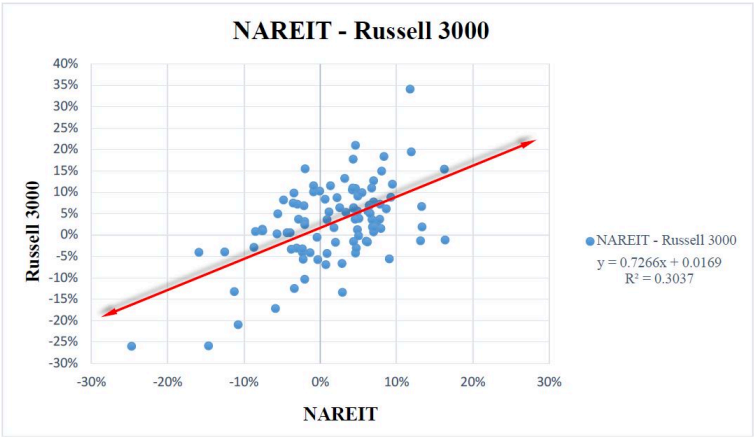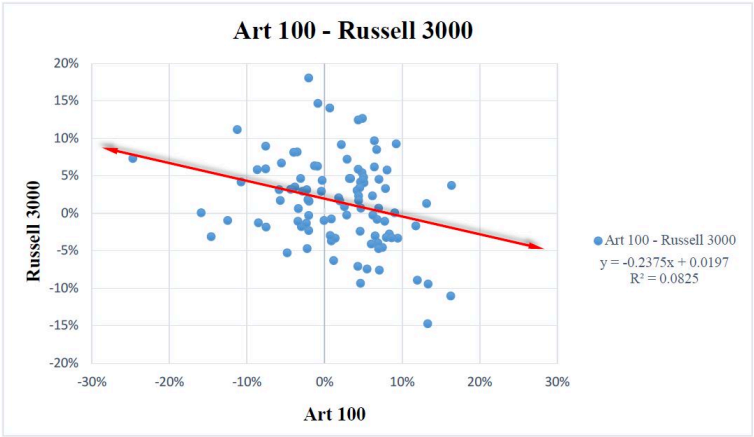
References

Arena, J. (2022, February 16). Deutsche Bank's John Arena: 'Art lending is a simple proposition' (Interview by Euromoney). Euromoney.

Ashenfelter, O., & Graddy, K. (2011). Sale rates and price movements in art auctions. The American Economic Review, 101(3), 212-216.

Bandle, A. L. (2015). Fake or fortune? Art authentication rules

in the art market and at court. International Journal of Cultural Property, 22(2), 379-399.

Blackman, A. (2015, June 15). What's That Hanging on Your Wall? Call it Collateral: Banks increasingly are offering loans secured by borrowers. The Wall Street Journal, Eastern Edition.

Bruno, B., Garcia-Appendini, E., & Nocera, G. (2018). Experience and brokerage in asset markets: Evidence from art auctions. Financial Management, 47(4), 833-864.

Cash in on your Picasso; Art-secured lending. (2019, July 6). The Economist (London), 58.

Fairfield, J. (2022). Tokenized: The law of Non-Fungible Tokens and unique digital property. Indiana Law Journal, 97(4), 1261-1313.

Etro, F., & Stepanova, E. (2021). Art return rates from old master paintings to contemporary art. Journal of Economic Behavior & Organization, 181, 94-116.

Freddie Mac. (2023, April 6). [Primary Mortgage Market Survey] [Fact sheet]. Mortgage Rates.

Lack, J. (2016). LTV, Loan to Value. In For Rent By Owner: A Guide for Residential Rental Properties (p. 47). Atlantic Publishing Group.

Li, Y., Ma, X., & Renneboog, L. (2022). In Art We Trust. Management Science, 1-30.

Łydżba-Kopczyńska, B., & Szwabiński, J. (2022). Attribution markers and data mining in art authentication. Molecules, 27(1).

Medelyan, V. (2014). The art of a loan: When the loan sharks meet Damien Hirst's '$12-million stuffed shark'. Pace Law Review, 35(2), 643-660.

Mei, J., & Moses, M. (2002). Art as an investment and the underperformance of masterpieces. The American Economic Review, 92(5), 1656-1668.

Neuhaus, N. M. (2015). Art lending: Market overview and possession of the collateral under Swiss law. Art, Antiquity, and Law, 20(2), 145-155.

Pearson, J. L. (2015, March 25). Establishing clear title to works of art (Art, Auctions and Antiquities). Wealth Management.

Ray, K. (2015). Art and Cultural Property. The Secured Lender, 71(3), 16-21.

Sotheby's. (n.d.). The Value of Art [Video]. https://www.sothebys.com/en/series/the-value-of-art

St. Louis Federal Reserve. (2023). Market Yield on U.S. Treasury Securities at 30-Year Constant Maturity, Quoted on an Investment Basis [Fact sheet]. FRED.

The Washington Post. (2016, May 7). Case in Point: The fine art of financing art.

Weinberg, N. (2017, February 2). In JPMorgan's 'War Room,' Private Banking meets cross-selling. Wealth Management.

Yu, B., & Gastwirth, J. L. (2010). How well do selection models perform? Assessing the accuracy of art auction pre-sale estimates. Statistica Sinica, 20(2), 837-852.

Zhukova, A., Lakshina, V., & Leonova, L. (2020). Hedonic Pricing on the Fine Art Market. Information (Basel), 11(5), 252.

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4. The Intersection of Entrepreneurship and Latinx Critical Theory: An Analysis of Revenue Performance and Loan Approvals of Construction Contractors in Utah

Luis Ramirez

Faculty Mentor: Lyda Bigelow (Entrepreneurship and Strategy, University of Utah)

Disproportionate outcomes exist in entrepreneurship based on the identity of the founders. While the Hispanic/Latinx population accounts for 19% of the United States population, Hispanic/Latinx owned businesses account for only 5.8% of all businesses. We are especially interested in understanding how a Latinx identity impacts entrepreneurial outcomes in the low-barrier industry of construction. Since many studies explore the obstacles and barriers in education for Latinx students, this paper explores the challenges in accessing capital financing in the form of credit. This study collects the contact information of over 27,000+ licensed construction contractors from the Utah Department of Occupational and Professional Licensing to distribute a IRB-approved 28 question Qualtrics survey. The survey collects firm data and owner demographic data. The survey data is analyzed with Python programming language to run descriptive statistics and regression analysis on revenue performance and bank loan approvals. Our survey received a survey response rate of 7.2 percent (n=1,974). The results from the survey showed that there is a disparity between the percent of Latinx contractors in Utah (12.5 percent) and the loan approval rates of Latinx for business loans from banks and credit unions (7.2 percent) (figure 1.). The regression (figure 2.) on annual revenue performance (r-squared = 0.55) found that contractors generated $40k more if they had a business loan from a bank (p-value = 0.07), $180k more if they had a PPP loan (p-value=0.00), $76k more if they were a general contractor instead of subcontractor (p-value=0.00), $2k more per year of experience (p-value=0.039), $39k more if they had

a business plan (p-value=0.059), $36k more per employee hired (p-value=0.00), $98k more if their accountant is CPA (certified public accountant) certified (p-value=0.001), $152k if they have a C-Corp instead of L.L.C. or sole proprietorship (p-value=0.081), and $52k more if they have a bachelors degree (p-value=0.021). The regression (figure 3.) on loan approval rates (r-squared=0.28) found that contractors loan approval rates increased by 0.19 percent per year of experience (p-value=0.02), 5.2 percent if they have a business plan (p-value=0.01), 18.7 percent if they previously qualified for a SBA loan (p-value=0.00), 4.7 percent if they have a bachelor's degree (p-value=0.03), and 4.2 percent if they focus on commercial instead of residential construction (p-value=0.06). The regression on loan approval rates also found that the bias of the loan officer (p-value=0.00) can impact loan approval rates by up to 29.5 percent, and English as a first language (p-value=0.02) decrease loan approval by 9.9 percent. The results from the findings prove that banks and credit unions must mitigate the bias of loan officers and create equitable credit assessments for Latinx entrepreneurs on construction companies. Furthermore, construction contractors can increase their annual revenue performance and loan approval rates by becoming general contractors, hiring more employees, upgrading to a C-Corp if they have an L.L.C., having a CPA-certified accountant, borrowing more money, and completing a bachelor's degree.

Figure 1: Bank and Credit Union Loan Approvals by Race (Percent)
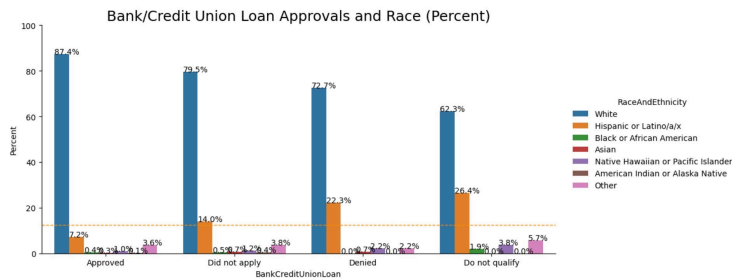
Figure 2: Regression on Revenue

Figure 3: Regression on Bank Loan Approval

```
                              OLS Regression Results
==============================================================================
Dep. Variable:      BankCreditUnionLoan   R-squared:                      0.286
Model:                              OLS   Adj. R-squared:                 0.276
Method:                   Least Squares   F-statistic:                    31.15
Date:                Wed, 26 Apr 2023   Prob (F-statistic):          5.94e-123
Time:                        02:19:43   Log-Likelihood:               -1026.4
No. Observations:                1974   AIC:                            2105.
Df Residuals:                    1948   BIC:                            2250.
Df Model:                          25
Covariance Type:            nonrobust
==============================================================================
                         coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------
const                  -0.3293      0.105     -3.151      0.002      -0.534      -0.124
LicenseClassification   0.0138      0.021      0.649      0.516      -0.028       0.055
Gender                  0.0384      0.028      1.371      0.171      -0.017       0.093
CreditScore             0.0003      0.000      2.404      0.016    5.79e-05       0.001
HomeOwner               0.0341      0.028      1.234      0.217      -0.020       0.088
EnglishLanguage        -0.0994      0.043     -2.327      0.020      -0.183      -0.016
YearsofExperience       0.0019      0.001      2.324      0.020       0.000       0.003
BusinessPlan            0.0526      0.021      2.561      0.011       0.012       0.093
EINApplication          0.0254      0.019      1.311      0.190      -0.013       0.063
NumOfEmployees          0.0024      0.002      1.338      0.181      -0.001       0.006
WorkAccidents           0.0150      0.007      2.146      0.032       0.001       0.029
SBALoan                 0.1879      0.025      7.518      0.000       0.139       0.237
PPPLoan                 0.0933      0.023      4.003      0.000       0.048       0.139
LoanOfficerRelationship 0.0590      0.006     10.579      0.000       0.048       0.070
AccountantCPA           0.0292      0.028      1.034      0.301      -0.026       0.084
Revenue2022          3.923e-05   2.21e-05      1.772      0.077   -4.19e-06    8.27e-05
TotalOutstandingDebt    0.0002   2.83e-05      5.743      0.000       0.000       0.000
SoleP                   0.0185      0.036      0.517      0.605      -0.052       0.089
Corp                    0.0342      0.022      1.560      0.119      -0.009       0.077
LLP                     0.0571      0.063      0.901      0.368      -0.067       0.181
Bachelor                0.0472      0.022      2.115      0.035       0.003       0.091
Master                  0.0312      0.035      0.904      0.366      -0.037       0.099
Latinx                 -0.0841      0.042     -1.980      0.048      -0.167      -0.001
Commercial              0.0428      0.023      1.856      0.064      -0.002       0.088
Industrial              0.0750      0.052      1.454      0.146      -0.026       0.176
Infrastructure         -0.0054      0.049     -0.110      0.913      -0.102       0.092
==============================================================================
Omnibus:                      174.782   Durbin-Watson:                  2.001
Prob(Omnibus):                  0.000   Jarque-Bera (JB):              90.822
Skew:                           0.365   Prob(JB):                    1.90e-20
Kurtosis:                       2.244   Cond. No.                    1.41e+04
==============================================================================
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5. # Coming of Age: Young Investors and the Rise in Riskier Investments

Kacey Tollefson

Faculty Mentor: Nathan Seegert (Department of Finance, University of Utah)

Abstract

By the year 2034, Generation Z (currently those between 12 and 25 years of age) is projected to become the largest generation with an estimated 78 million individuals (Morgan Stanley, 2014). In all respects, this rising generation is taking the world by storm- especially in the investment sphere. Not only are there more young investors than any generation that proceeded them but they're also more likely to take risks- knowingly or unknowingly. A basic framework for making

an investment decision includes defining one's investment objectives, researching opportunities, and allocating funding across selected assets. While Gen Z is just as motivated to save for retirement as the older age groups, they're also the most interested in getting rich. Influenced by their heavy utilization of social media, young people are making investment decisions contrary to sound financial principles; this includes taking on debt to invest and by buying assets with arguably no intrinsic value. On the contrary, this generation is expected to be the most educated with the highest probability of having college educated parents. They are also consuming less alcohol and have less teenage pregnancies than previous generations (Hawkins et al., 2022). Millennials and Gen Z are investing in retirement at a younger age than their parents and grandparents and strive for financial independence. Consequentially, many young investors are doing their own investment research as opposed to using index-based mutual funds or by working through financial advisors. While many would argue that financial markets are efficient, these 'young guns' don't believe that's the case and are willing to make trades against it. Their extensive involvement in high frequency trading has led several behavioral finance professionals to correlate it with gambling, especially as young investors seek high short term yields as opposed to consistent investing. This paper will analyze how the evolution of equity market trading has allowed for increased access to markets in modern times. It will also relate generational differences between investors to compare risk tolerance and investment preferences.

Keywords: Generation Z, risk tolerances, behavioral finance, high frequency trading, investment strategies and investment objectives

Introduction

Strategic investing has long been regarded as a wise tool for both wealth creation and building generational wealth. Participation not only allows one to prepare for future expenses but also as a hedge against risks such as inflation. Prior to the 1980's, market participants were comprised of institutional investors and individuals with extensive educational backgrounds. The process of exchanging assets was complex and often limited participants to certain geographical areas. The invention and adoption of digital technology simplified this process and allowed more investors to participate.

The transition from a small number of investors to the majority of Americans didn't happen overnight. As of 1900, only about 1% of Americans had equity investments, but by 2021 this proportion increased to 55%, (as shown in Figure 1). This shift was fueled by economic prosperity, financial innovations, and better data transparency.

Figure 1: Percentage of Americans Invested in Stocks

Figure 1: Percentage of Americans Invested in Stocks

Data source: https://www.pbs.org/fmc/book/14business6.ht
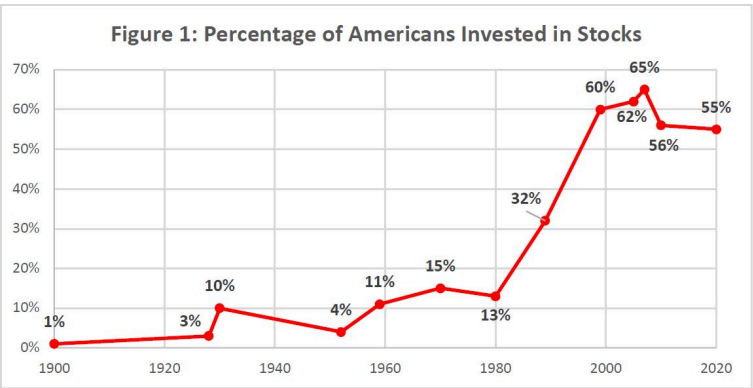
Beginning in the late 1800s, railroad companies were some of the first few American institutions to issue stocks and bonds

(Beattie, 2023). These assets were fairly illiquid and were only held by wealthier clients seeking long-term growth, or about one percent of the entire United States population. This proportion would later grow to a high of 10% in the late 1920s due to economic success and larger disposable incomes.

According to the U.S. Census Bureau (2021), America's economy grew 42% in the 1920s. Innovations such as the radio spread information much faster than the traditional newspaper, updating the public with economic or financial news in real time. More than 25% of families had their own car and airplane travel was up 287% (Armadeo, 2022). In 1928, stock prices rose 39% following a five-year bull run that began in 1923. Unfortunately, this long-term market success was severely corrected on October 29, 1929, (otherwise known as "Black Tuesday"), when billions of dollars of wealth were wiped out in a single day, effectively ushering in the Great Depression. This economic decline coupled with a lot of distrust and speculation about the market reduced the proportion of Americans with stock back down to 4.2% by 1952.

It was not until 1954 that the Dow Jones Industrial Average recovered to its pre-depression levels. With the introduction of Standard and Poor's 500 index (S&P 500) in 1957, mutual funds grew in popularity over the next several years as it allowed a better way for previously hard-hit investors to diversify their holdings (Anderson, 2021). On February 8, 1971, the NASDAQ exchange was launched as one of the world's first electronic stock markets. These improvements increased investor access to financial markets, raising American participation rates to an all-time high of 15% in 1970 before eventually settling back down to 13% in 1980.

The following two decades saw strong economic growth with the S&P 500 increasing 17.4% annually as opposed to its

historical 9.7% annual return (Vise, 1989). Despite the political tensions of America's involvement in foreign affairs, trust in the markets remained high, especially as inflation rates dropped from 15% in 1980 to 3% by 2000 (U.S. Bureau of Labor Statistics, 2021). According to the Economic Policy Institute, it wasn't until the third quarter of 2001 that the economy experienced its first contraction since 1993 (Weller, 2002). These movements and conditions subsequently increased equity market participation from 32% in 1990 to a high of 60% in 2000.

As of 2020, it is estimated that 55% of Americans are invested in stocks. Over time, periods of strong economic growth and technological advancements have proven to increase participation in the market, and the same holds true for our currently young, rising generation. Several new brokerage firms such as Robinhood and Public offer equity and crypto market trading with no transaction fees. The obsession over social media as a news source has increased financial awareness not only for stocks but for alternative investments such as cryptocurrency and derivatives trading.

According to Fortune magazine, "Today's millennials and Gen Z have more economic power than any generation that preceded them. They are earning more, saving more, and investing earlier and at a higher rate than previous generations" (Case, 2021). These younger generations are eager to prepare for the future by starting earlier. A recent study by the CFA Institute (2022) found that 33% of Gen Z and 31% of millennials made their first investments before 21 years of age, presenting a statistically higher level of participation than preceding generations, with Generation X at 14% and Baby Boomers at 9%, (see Figure 2). For purposes of this study, those belonging to Gen Z are considered those born between 1997 and 2012, millennials are born between 1981 and 1996,

Generation X are born between 1965 and 1980, and Baby Boomers are born between 1946 and 1964.

Figure 2: % Generation Invested by 21 years old

Figure 2: % Generation Invested by 21 years old
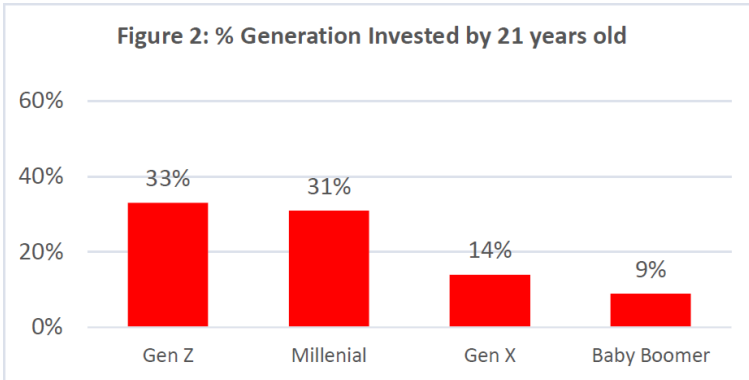
Data source: Fortune magazine, based on a NASDAQ study

While saving and investing early on has its advantages, making those decisions prematurely without a sound understanding of financial markets or their associated risks is worrisome. Both Baby Boomers and Gen X were raised by parents who endured the Great Depression, and according to Spectrem Insights' study (2014), this generation that "came of age during the economic collapse and subsequent recession," has developed a more cautious risk tolerance. Conversely, most of Gen Z has not experienced financial challenges or investment risks and as such may have a higher risk tolerance.

As per FINRA's 2018 report, parents are engaging in more investment-related discussions with their children than before. While this does not imply a causal link as to why younger people are investing more, it does highlight growing financial awareness among the younger generations. This study will examine whether the young generation's increased level of participation and investment decisions are demonstrating a higher risk tolerance than those who preceded them.

Methods

The younger generations are being brought up in a world of technology. The youngest member of Gen Z was only 10 years old when the first iPhone came out. Information is now available at the touch of a button, and commission free trading platforms make it much cheaper for individuals to invest. Social media has become a powerful tool for information and sharing ideas, especially in the investment space. All of these factors are influencing a generation to act and invest sooner than any that have proceeded them.

This research focuses extensively on behavioral finance and risk tolerances between Gen Z/millennials and those belonging to the Gen X/Baby Boomer generations. This will be accomplished by using an investment decision making framework of (1) defining investment objectives, (2) data and information collection, and (3) asset allocation.

Investment objectives can be defined as the goals or strategies an investor has for entering into an investment. These goals might include preparing for retirement or saving up to buy a house. Data and information collection is the process of performing technical research and valuations of different investment opportunities that will help an investor achieve their investment objectives. Asset allocation involves buying, selling, and balancing one's portfolio of assets to maximize returns while reducing risks. A consistent strategy entails aligning all three phases of this process.

For example, an investor preparing for retirement may elect for more volatile assets such as stocks in the beginning. As the investor gets closer to retirement, it is generally advisable to move towards safer, less volatile assets such as bonds to create a more stable portfolio value (Beagle, 2023). In this study, we will relate each of these phases between the younger and older

generations to observe differences in investment objectives and motivations.

This study is being conducted under the hypothesis that young investors aren't as financially literate as their predecessors and are therefore making riskier investment decisions. Several areas we will investigate for this generational comparison include their trusted or most used sources for financial advice, the length of time spent studying investments, how often they monitor their portfolios/what's in them, how frequent they trade, and differences in money sources being used to invest.

Results

Investment Objectives

An investor seeking to generate additional wealth through investing will typically do so with an end goal in mind. This can include buying a house, saving for a child's college fund, retiring or even just to build generational wealth. These incentives often influence the type of assets one should buy and how long to hold them for. For this reason, an investor's time horizon and risk tolerance are taken into consideration when building an investment objective. The longer an investor has before their targeted return date, the more likely they're able to benefit or recover from riskier portfolios.

As it turns out, every age cohort's main objective for investing is to eventually retire. A recent 2022 study conducted by MagnifyMoney found that of the 670 investor respondents, 62% of them wanted to retire comfortably. Figure 3 illustrates that although this goal is at a majority across all generations, its prioritization grows the older an investor gets (Cook, 2022). Interestingly enough, the next highest motivation for investing among Gen Z and millennials was to get rich, at 29% and 22%, respectively.

Figure 3: Primary Investment Objective of Americans

Figure 3: Primary Investment Objective of Americans
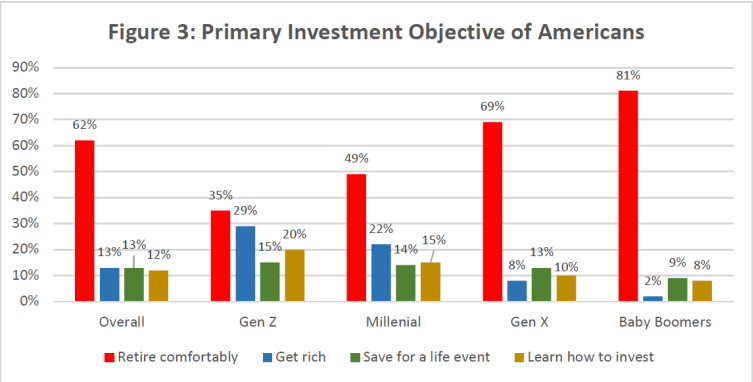
Data source: https://www.magnifymoney.com/news/ investing-by-generation-survey/ total may not add to 100% due to rounding

Understandably young investors may not be as concerned about retirement given that its farther away from them, but the study also highlighted how Gen Z and millennials are also interested in learning how to better invest. Given that such a higher proportion of these young individuals are investing than before, it's likely that they don't have financial experts or colleagues to consult with and are therefore learning to invest on their own. Their motivations, though, are more mixed.

Another study conducted by The Royal Mint, the United Kingdom's currency manufacturer, supported the findings of the adolescent "get rich quick" mentality. Their study found that 17% of respondents started investing after seeing substantial investment successes on social media, which unfortunately turned out to financially worsen many of them (The Royal Mint, 2022). Nearly two thirds of their respondents aged 16-25 years old reported severe losses, so much so that many of them are now focusing on diversification than picking the next big stock.

These findings suggest that although retirement is of importance to Gen Z, they're also willing to take a lot of chances and learn through experience. Their relatively young age affords them more time to recover from potential risks, especially as they learn wise investment principles such as the value of asset diversification.

Data & Information Collection

Gone are the days of reading the newspaper or having to ask your neighbor what they heard on the radio. TikTok, Instagram, Facebook and Snapchat are all the new norms of staying involved and up to date. These free platforms allow users to build a network of trusted family and friends while also exposing them to popular channels or influencers. These influencers have a significant online following and usually gain the trust of those who follow them. Their social status affords them influence on their followers such that they are highly persuasive on things such as purchasing products or making investment decisions.

According to a 2021 Motley Fool survey with 1,400 respondents between the ages of 18 and 40, social media ranked as the highest information source for making investment decisions. When asked, "Please select the types of resources used to get information on investing in the past 30 days," 91% of Gen Z and 75% of millennials selected social media as opposed to 41% for friends and family (Caporal, 2021). Figure 4 illustrates that when it comes to risking one's own money, young investors are more likely to rely on social media stars they've never met in-person than their own familial relationships.

Figure 4: Sources Used for Investment Decisions Gen Z Millenial Males Females

Figure 4: Sources Used for Investment Decisions

Data source: https://www.fool.com/research/gen-z-millennial-investors-tools/
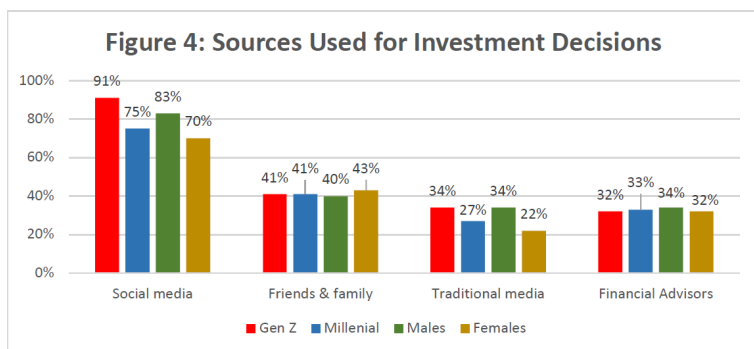
The inherent danger of relying on social media as an information source is that those giving financial advice aren't accredited to do so. Financial planners are individuals who help manage your investment portfolio, and although not required to gain accreditation and certification, many of them do so to both improve their credibility and expertise. A Certified Financial Planner must undergo extensive educational and ethical training in addition to thousands of hours of financial planning experience before they can get certified.

For financial social media influencers, or "Finfluencers", their credibility can be easily manipulated through the number of video likes or followers they have. Popular advertisements such as, 'How I made $X in 30 minutes' are eye-catching for inexperienced investors who might not be financially well off. This leads these individuals to invest on the illusion of getting high future returns even though it's based off of an influencer's performance in the past.

Take Kim Kardashian, for example. In June of 2021, without disclosing her $250,000 incentive from EthereumMax, Kim promoted their cryptocurrency to her then 220 million followers on Instagram (Sweney, 2022). Sixteen months later

the coin had lost 95% of its value and Kim was hit with a $1.26 million fine from the SEC for failing to disclose she was being paid to post her recommendation (Hartmans, 2022). While no one can definitely say whether an investment will be successful or not, popular influencers or celebrities commandeer a lot of trust from the public in what they recommend doing.

In terms of the most popular social media sources used for making investment decisions, the same Motley Fool survey found that 71% of Gen Z respondents used YouTube, 42% used Reddit, and 36% used TikTok (Caporal, 2021). Millennials yielded similar results with 52% using YouTube, 36% using Reddit, and 35% using Facebook (see Figure 5).

Figure 5: Social Media Sources Used for Investment Decisions Gen Z Millenial Male Female

Figure 5: Social Media Sources Used for Investment Decisions

Data source: https://www.fool.com/research/gen-z-millennial-investors-tools
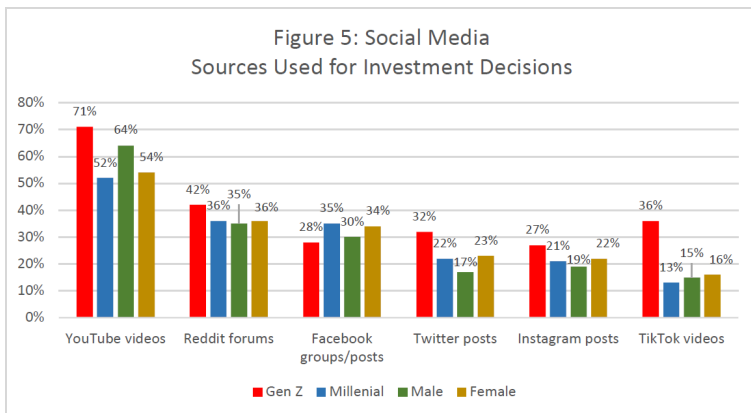
Due to several laws limiting financial advice giving to accredited professionals, many YouTube channels or livestreams have the disclaimer "this is not trading advice" when talking about investment strategies. Unfortunately, however, falling into the trap of trusting these channels due

to illusory credibility is far too common. Take Chris Boutte, a young author and social media influencer himself, for example.

In 2021, Chris decided to become a "financially responsible adult by investing and saving for [his] future" (Boutte, 2021). After receiving a few thousands of dollars as an inheritance, Chris turned to something he was familiar with- YouTube- to learn what to do with it. "Many of the YouTubers I watched are millionaires, so I assumed they knew what they were talking about. I was drawn by the belief that these people were experts." Unfortunately for Chris, this trust cost him about $3,300.

After listening to several YouTubers advocate that Coinbase's upcoming IPO shares should be valued at $700, Chris convinced himself not to buy, even though shares started trading at only $381. After rising to $429, he started to regret his decision; but once they dropped back down to $333, he said, "I figured I'd be a fool if I didn't buy. I bought three shares, and I've been losing money ever since."

While professional financial advisors can certainly get things wrong as well, Chris' mistake was getting caught up in the "fanaticism" of YouTube on certain stocks. After he started incurring losses, Chris went back to see just how many of his favorite YouTube channels were right, but not many of them were. "I learned the expensive lesson that these YouTube "experts" should only be watched for entertainment purposes, and that's about it," Chris concluded.

While Gen Z and millennials are going to more untraditional sources for investment advice, many of them still trust financial advisors the most. According to a recent NASDAQ survey, financial advisors were ranked the highest in terms of trustworthiness (NASDAQ, 2022). Although millennials and

Gen Z rely heavily on social media, they trust online forums and podcasts more (see Figure 6).

Figure 6: Trusted Sources of Information

Figure 6: Trusted Sources of Information
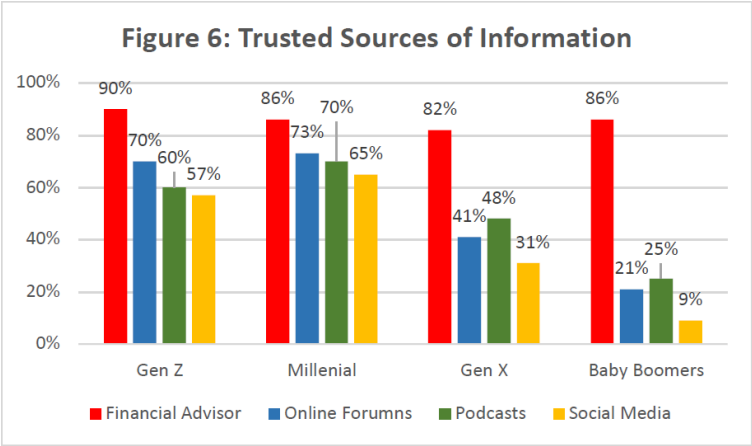
While these young investors may trust credible advisors the most, they're also the least likely to use them. This doesn't mean they aren't doing their own research, however. Based on the same 2021 NASDAQ survey, Gen Z and millennials are actually spending the most time before buying/selling an asset (see Figure 7). According to the survey, 40% of Gen Z respondents spent at least one hour to one day researching with the proportion declining the older the generation gets (NASDAQ, 2021). In terms of researching one day to one week, Gen Z, millennials, and Gen X have similar proportions near 30%, with Baby Boomers at only 18%. A quarter of Baby Boomers don't do research at all before investing, but this could be a result of their investment experience or financial advisors doing the research for them.

Figure 7: Time Spent Researching before Buying/Selling

Figure 7: Time Spent Researching before Buying/Selling

Data source: https://www.nasdaq.com/docs/etps-empowering-next-gen eration-of-investors
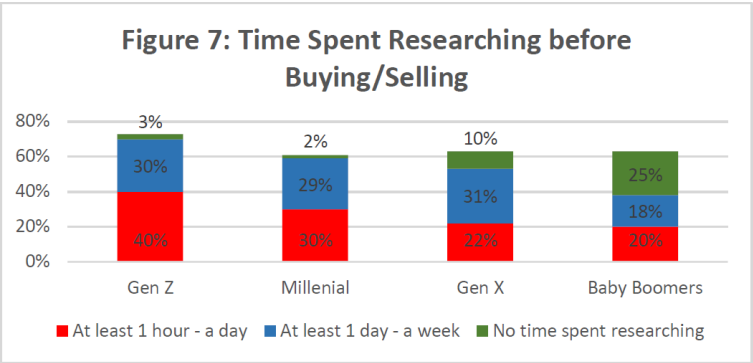
Not only are Gen Z and millennials highly reliant on their own research but they are also obsessed with the performance of their investments. Whether that be out of eagerness for short-term, high yields or a lack of confidence, 48% of Gen Z and 39% of millennials check their portfolios several times a day (see Figure 8) (NASDAQ, 2021). This proportion falls by over half with only 16% of Gen X and 10% of Baby Boomers. On average, 19% of all age categories check their portfolios at least once a day, but young investors in particular are interested in more frequent monitoring.

Figure 8: How Often do you check your investment portfolio

Figure 8: How Often do you check your investment portfolio

Data source: https://www.nasdaq.com/docs/etps-empowering-next-gene ration-of-investors
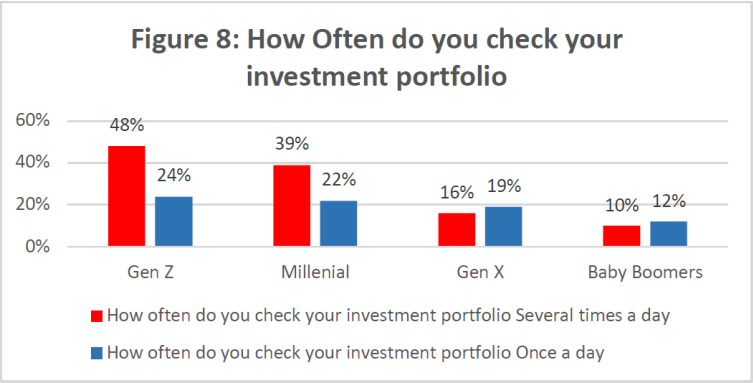
In conclusion, younger investors aren't afraid to turn to untraditional sources of information for investment advice. While they hold financial advisors as the most credible source in line with other generations, social media remains the highest used source. While the NASDAQ survey did not specify whether the time spent researching assets entailed any sort of technical analysis, young investors are obsessed with seeing how well they're doing- or how much they've lost.

Asset Allocation

Historical finance classified investment assets as one of three different domains. These included equities, fixed income, and money market instruments. Equity investments, or buying and selling stock, have become more complicated with the introduction of options and derivatives. Options, or derivatives, provide a way to hedge, (or bet), on a stock's future performance, whether it be an appreciation or depreciation in value. While not specific to equities, the value derivatives are contingent upon the price of the underlying asset or stock, (i.e., your option is only as valuable as the stock price's movement).

Investing in fixed income investments entails buying or selling bonds, or debt instruments issued by companies or the government. United States Treasury bonds are frequently referred to as "riskfree" investments due to a perceived low risk of government default. Corporations and municipalities are also able to issue debt, however, fixed income assets usually don't yield as high of returns as equity investments due to lower risk of default. In the event of a bankruptcy, debt investors are held at a much higher priority than equity investors, and most fixed income instruments are backed by a specific asset.

Money market instruments are short-term assets such as treasury bills, commercial paper, municipal notes, and certificates of deposit (or CDs). These assets are usually purchased at physical locations such as banks or directly from the government entity's website. Due to the low risk, short term nature of these investment assets, they are also referred to as "cash or cash equivalents."

More recently, alternative investments such as real estate, cryptocurrency, commodities, and derivatives have grown in popularity. Cryptocurrency is a form of digital currency stored by a decentralized system. When Bitcoin, (one of the first cryptocurrencies available), launched in January of 2009 at a price of $0.0008 USD, many people didn't take notice. It wasn't until 2011- 2013 that the coin started getting noticed, especially as it rose to a price of $64,800 per coin on April 14, 2021.

While the rest of the world saw a high value in Bitcoin, several experienced investors did not. Warren Buffet, CEO and chairman of Berkshire Hathaway, (whos portfolio value is calculated at around $299 billion), stated that, "Whether [Bitcoin] goes up or down in the next year, or five or ten years, I don't know. But the one thing I'm pretty sure of is that it doesn't multiply, it doesn't produce anything. It's got a magic to it and people have attached magic to lots of things" (Macheel, 2022). Many financial experts have argued over the intrinsic value of cryptocurrency or the lack thereof, and while this debate doesn't fit the merits of this paper, it's worth noting that cryptocurrency has demonstrated itself as a highly volatile asset whose true worth lies in the eyes of the market.

What does this mean for Gen Z investors? As it turns out, cryptocurrency is the second highest investment owned at 47%, proceeded only by stocks at 73% (Figure 9) (NASDAQ, 2021). The enormous uptick in Bitcoin price appreciation and its

coverage on social media likely exposed these youth to investing for the very first time. This was the case for Chris Boutte who, like many of his peers, saw this potential for high reward as one you wouldn't want to miss out on.

Figure 9 Investment Securities owned by Gen Z

Figure 9: Investment Securities owned by Gen Z

Data source: https://www.nasdaq.com/articles/gen-z%3A-what-to-know-about-the-next -generation-of-investors
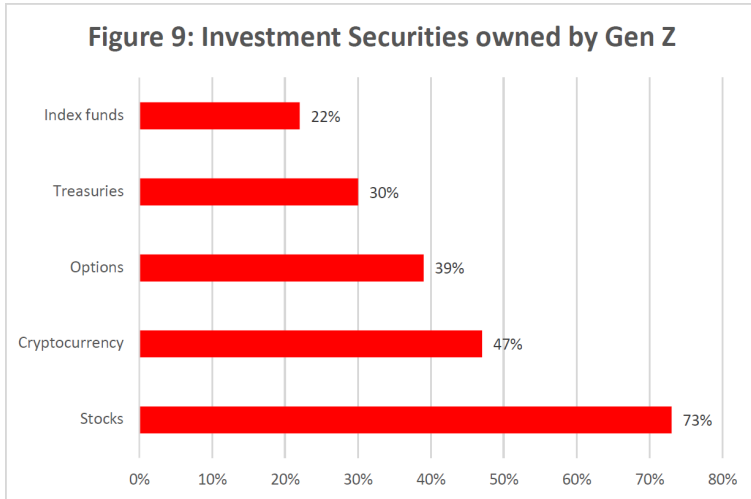
Despite Gen Z being a significant market participant in cryptocurrency, they also exhibit high levels of interest in options trading. Whether through puts, calls, forwards or futures, participating in options is often referred to as a zero-sum game- when one investor wins big, the other investor loses by the same amount. Selling call options in particular can be dangerous for an unsophisticated investor due to the uncapped potential of losses, but this generation has an alarmingly high level of participation in it.

Fortunately, however, 30% of Gen Z investors are invested in United State Treasuries, which in some way allows them to buy down some of the investment risks they're participating in.

In terms of equity investments, however, they're also the least likely generation to use index funds.

According to both a MagnifyMoney survey and a Motley Fool study, Gen Z respondents were the least likely to be invested in index/mutual funds at 26% and 35%, respectively (Cook, 2022; Hawkins et al., 2022). Baby boomers were the most likely to be invested at 57%, followed by 38% of Gen X and 31% of millennials (Cook, 2022). A mutual fund is a collection of assets pooled together and sold in terms of shares, allowing investors to diversify idiosyncratic risk in a costefficient, hassle-free way.

According to data published by the United States Federal Reserve in Figure 10, overall investments in mutual funds have risen at a compound annual growth rate of 16.16% since 2009. The Federal Reserve also provided data for those who were under 40, 40-54, 55-69, and 70+ years of age, wherein they found that those under 40 had the lowest CAGR of 12.18% (Federal Reserve, 2022). While many belonging to older generations are investing more money into mutual funds, the younger generations are not. Instead, younger investors are more interested in picking their own assets or stocks to buy.

Figure 10: Corporate equities and mutual fund shares by age

Corporate equities and mutual fund shares by age

Source: Survey of Consumer Finances and Financial Accounts of the United States
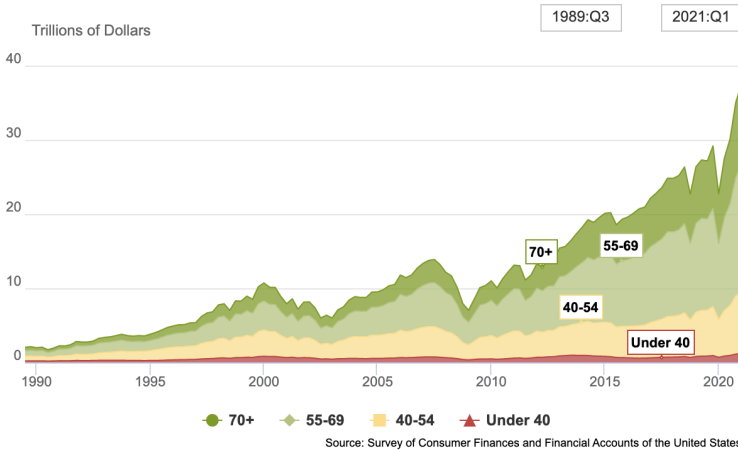
Data source: https://www.federalreserve.gov/releases/z1/dataviz/dfa/ distribute/ chart/#quarter:126;series:Corporate%20equities%20and%20mut ual% 20fund%20shares;demographic:age;population:1,3,5,7;units:lev els;range:2010.1,2 021.1

By not relying on mutual funds or ETFs, investors have to take a more active approach in selecting which assets to buy and when to sell/rebalance their portfolio. Mutual funds are managed by portfolio managers who handle all of those administrative details for a fee, but an investor wanting to make those decisions for themselves will likely seek control of their portfolio by cutting out the third-party. How frequent an investor wants to do this demonstrates which strategy they are pursuing.

Two strategies investors can take are buy-and-hold and market timing. According to U.S. Bank (2020), a buy-and-hold strategy is a "passive, long-term investment strategy that creates a stable portfolio over a long period of time to generate

higher returns." A passive investor will only periodically reevaluate their portfolio, ignoring short term losses for more favorable gains in the long term. A much more active investor participates in market timing, or closely following the market to try and identify bull or bear trends. These investors are seeking above-average returns in an attempt to build wealth quickly.

A recent academic article published by Philip Newall and Leonardo Weiss-Cohen highlights the correlation between investor attributes and beliefs to holding strategies. "The two main strands of financial economics," they write, "both recommend that the average investor should rarely buy and sell stocks, known collectively as 'trading'" (Newall et al., 2022). Over time, research has found that long-term, buy-and-hold strategies consistently outperform market timing. By investing for the long term, an investor is playing a positive sum game- as a company performs well, profits increase, thereby allowing the company to pass those returns on to investors in the form of dividends or stock price appreciation.

When an investor is trying to time the market, they believe that the market is somehow inefficient; that the current price of an asset doesn't fully reflect its value. By consistently trying to buy at a low and sell at a high, the investor has to be right twice in order to be successful. Newall's research has found high frequency trading to be more of a zero-sum game, where no wealth was actually created but instead redistributed from the investor who was wrong to the investor who was right.

According to a twelve-year timespan of trading data in Newall's research, high frequency traders were profitable only 5% of the time. While these traders earn above market returns, the chances of success do not favor the average investor. Long-term investing, on the other hand, leads to an average

profitability of 10% annually. In terms of investor attributes, Newall found that long-term traders were patient and objective and often focused more on strategic analysis. High frequency traders, however, were overconfident, impulsive, and sensation seeking.

One valid concern raised by Newall's research is the gamification, or "gamblification," of stock trading, and just how relatable high frequency trading is with gambling. Casino gambling, for example, does not favor the average better in the long term. Significant investments are made by the casinos to get customers to stay longer, knowing full well that a gambler is more likely to lose the longer they play. It is also a zero-sum game- either you win or the house wins.

Furthermore, gamblers and high frequency traders have demonstrated similar behavioral patterns. Not only are their attributes similar in terms of overconfidence and sensation seeking but Newall also found that, "People experiencing gambling-related harm usually show signs of behavioral dependence, in that they spend more time engaging in gambling and thinking about gambling than they would prefer" (Newall, 2022). This level of "gambling-related harm" isn't far off from Gen Z, wherein they're not only spending the most time researching investments but they're also the age category making the most trades a week (see Figure 11).

Figure 11: % Making Multiple Trades a Week

Figure 11: % Making Multiple Trades a Week

Data source: https://www.nasdaq.com/docs/etps-empoweringnext- generation-of-investors
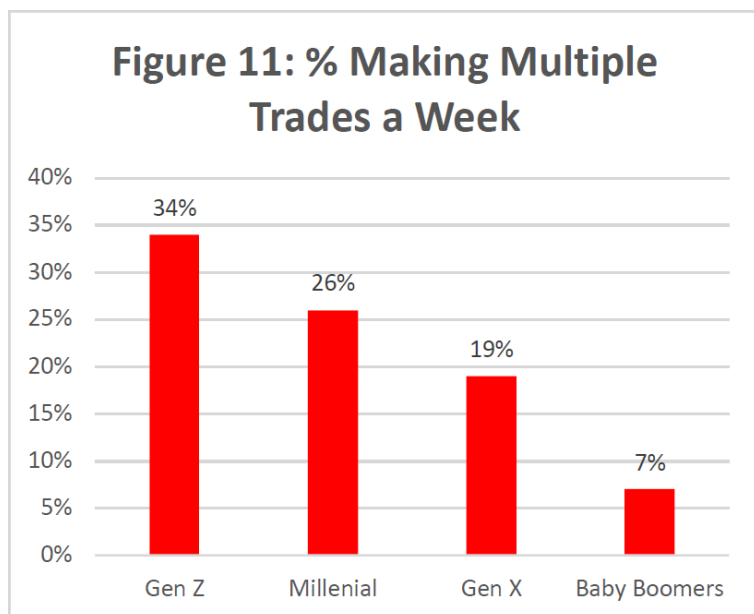
Even though research has shown that frequent trading is correlated with worse returns, Gen Z are preferring it over a buy-and-hold strategy (Bonaparte, 2010). Part of this reason may be due to the trading platforms they use. Many of the trading apps such as Robinhood and Publix use gamification elements to increase investor activity; anywhere from confetti drops to notifications on trendy stocks. These platforms make money through order batch processing, which is contingent on having a high volume of trades. Using Robinhood is a lot like walking through a casino floor room— the flashing lights, dinging sounds, and free drinks for those at the table are meant to entice you to play more.

As despicable as it is to lose a lot of money at a casino, it's even worse when that money was borrowed. According to a MagnifyMoney survey of over 2,000 respondents,

approximately 80% of Gen Z investors have taken on debt to invest (Evans, 2021). While this is followed closely by millennials at 60%, it then drops down significantly to 28% for Gen X and 9% for Baby Boomers (see Figure 12). Of those who took on debt to invest, 46% claimed they've borrowed $5,000 or more, 22% borrowed less than $1,000, 32% borrowed $1,000 to $4,999, 26% borrowed $5,000 to $9,999, and 20% borrowed $10,000 or more (Evans, 2021).

Figure 12: % Generation who've taken on Debt to Invest

Figure 12: % Generation who've taken on Debt to Invest

Data source: https://www.magnifymoney.com/news/debt-to-invest/
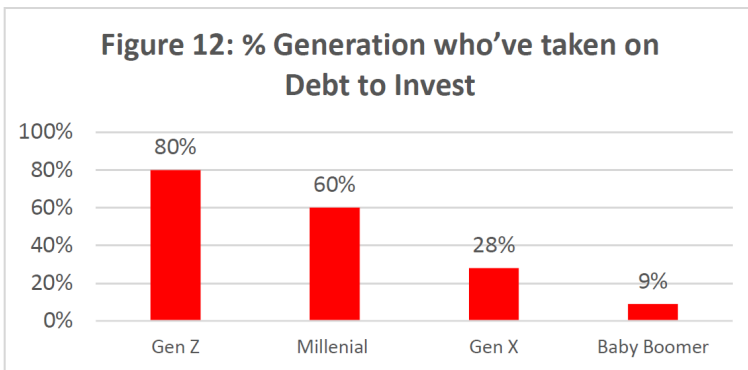
Not only are younger generations making risky investment decisions but they're also using debt to do so. This compounds the issue of experiencing average negative profits of high frequency traders. Investors will not only have to pay back the sum they lost in investing but they will also have to pay interest on it too, interest that likely started from when they first took on the debt.

Traditional financial literacy would advise one to pay off current debts before investing, but in this situation, investors are actually taking on debt to invest. MagnifyMoney also went into greater detail to find the sources of debt funding as well as

the primary purpose for taking on that debt (Figures 13 & 14). Of those who took on debt to invest, personal loans were the largest category at 38%, followed by friends and family at 23% and credit cards at 14%.

Figure 13: Source of Debt Funding

Figure 13: Source of Debt Funding

Data source: https://www.magnifymoney.com/news/debt-to-invest/
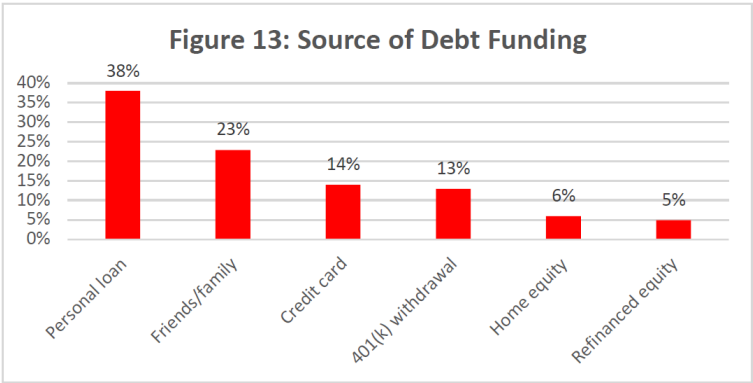
While many are taking on debt to invest, fortunately, the largest proportion of them are doing it for a good reason. 37% of those who took on debt to invest put it in a retirement plan, anticipating a higher, longer-term return than what the interest might be worth. However, 32% took on debt to buy a particular stock and 31% did so in order to participate in day trading.

Figure 14: Motive for Taking on Debt to Invest

Figure 14: Motive for Taking on Debt to Invest

- Put it in retirement plan — 37%
- Buy a particular stock — 32%
- Participate in day trading — 31%
- Buy cryptocurrency — 10%
- Other — 3%
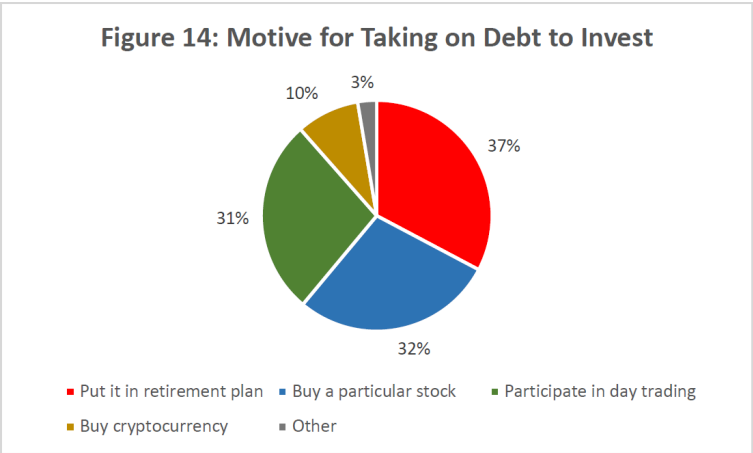
Data source: https://www.magnifymoney.com/news/debt-to-invest/, respondents were allowed to select more than one answer if applicable.

Conclusion

Through the use of several different studies and research databases, we were able to find that younger investors prefer a more DIY approach to investing and are willing, knowingly or unknowingly, to bear the risks of doing so. While all age groups main motivation for investing is to be able to afford a comfortable retirement, Gen Z and millennials are also motivated to elevate their financial status and become wealthy.

Contrary to their older parents or grandparents, young investors are using untraditional sources of information to learn about investment opportunities. While they find financial advisors the most credible, they're also the least likely to use them. As it turns out, a study by Ernst & Young found that "70% of women and millennial/Gen Z investors will likely fire their family's advisors," especially as they begin to inherit the trillions of dollars' worth of portfolios currently being held by their Baby Boomer grandparents (Case, 2021).

Rather than using financial experts, young investors instead rely primarily on social media and their own analysis/perception of the market when investing. Regardless, younger generations want to take an active approach in managing their financial futures, evident by the extent of time they spend studying potential investments as well as how frequently they check their portfolios.

While open mutual funds are simple to access and participate in, it isn't enough for Gen Z or millennials. These investors want to make asset allocation decisions for themselves and are often persuaded to do so by emotional reason as opposed to logic. Currently, the largest trading platform for Gen Z, Robinhood, is set up to take advantage of their emotions and encourage more frequent participation (Newall, 2022).

In conclusion, these motivations and sources of information are encouraging young investors to participate in untraditional or alternative assets such as cryptocurrency. While they're financially aware of more investment opportunities, they also defy traditional financial principles by taking on debt to invest. Their high-risk tolerance is also illustrated by their high participation in options trading at two times more than the average rate (NASDAQ, 2022). Options trading is something that is recommended only for sophisticated investors who know what they're doing, many of whom use complex hedges to limit their losses.

Do these findings suggest that young investors are investing without merit at all? Is all of their asset allocation or motivations for investing bad? Not necessarily. While Gen Z and millennials are the most likely to frequently trade, they're also doing the most research beforehand.

Based on a millennial report filed by the Bank of America

utilizing 1,900 survey respondents, one in four millennials have more than $100,000 in their savings accounts (Pepler, 2021). That same study found that millennials started saving for retirement at the age of 24 as opposed to 30 for Gen X and 33 for Baby Boomers. Their generation was also the most likely to know and understand how capital gains taxes work when they first started investing (Personal Capital, 2021).

In the end, Gen Z and millennial investors want a better financial future for themselves, and they're starting at a younger age to do so. While everyone wants to pick the next Apple or Tesla stock, this generation also wants to be financially independent. Based on the aforementioned Bank of America study, 74% of millennials frequently check their account balances, 55% track expenses, 46% pay their credit card bills in full, 31% plan a budget and 34% of those who do actually stick to it (Pepler, 2021).

While gamification elements on trading platforms remains a concern for the Securities and Exchange Commission (SEC), further research has found that investment experience has a negative effect on risk tolerance (Beyoud, 2022). A study conducted by Surabaya State University in 2019 found that while "financial literacy has a positive effect on risk tolerance, investment experience has a negative effect on risk tolerance" (Kusumaningrum, 2019). Their statistical research and findings demonstrate that financially literate investors are more likely to understand the risks of certain assets than inexperienced ones and prepare better by mitigating some of those risks. Investment experience, on the other hand, decreases an investors risk tolerance- especially if that investor learns first-hand just how much risk is involved with investing.

With a much larger proportion of young people investing than ever before, their behavior isn't untypical of an

inexperienced investor. Research has found that while they stock pick now, the more losses they incur the more likely they're going to seek for diversification. Perhaps similar to how a gambler knows 'when to walk away,' it will be interesting to see how Gen Z and millennial risk tolerances change as they gain investment experience.

In the end, the younger generation is a force to be reckoned with. Innovation and social media have prompted their participation in investing, and many of them are here to stay. The current United States population over 65 years of age is expected to increase to 21.6% by 2040, and as these Baby Boomers begin liquidating their portfolios for retirement, the markets will be even more influenced by the rising generations (U.S. Department of Health and Human Services, 2020).

The best thing for us to do, as an investor or not, is to be informed. The world is polluted with information, good and bad, and being able to wade through the muck and skepticism will make all the difference moving forward.

References

6 reasons to buy-and-hold stocks for long-term investing: U.S. bank. 6 Reasons to Buy-and-Hold Stocks for Long-Term Investing | U.S. Bank. (2020, July 28). Retrieved March 26, 2023, from https://www.usbank.com/investing/financial-perspectives/investing-insights/buy-and- hold-long-term-investment-strategies.html#:~:text=Buy%2Dand%2Dhold%20is%20a,them%20despite%20any%20ma rket%20fluctuation.

AFREPRELAX News. (2022, September 3). *How much time do people spend on social media and why?* Forbes India. Retrieved March 24, 2023, from https://www.forbesindia.com/article/lifes/how-much-time-do-people-spend-on-social- media-and-why/79477/

1#:~:text=Generation%20Z%20%E2%80%94%2016%2D24%20year
,and%20 40%20minutes%20for%20men.

Amadeo, K. (2022, March 28). *1920s Economy: What Made the Twenties Roar*. The Balance. Retrieved March 23, 2023, from https://www.thebalancemoney.com/roaring-twenties-
4060511#:~:text=One%20reason%20for%20the%20boom,the%20r emaining%2080%2D9 0%25.

Anderson, S. (2021, January 26). *Stocks then and now: The 1950s and 1970s*. Investopedia. Retrieved March 23, 2023, from https://www.investopedia.com/articles/stocks/09/stocks-
1950s-1970s.asp

Beattie, A. (2023, January 30). *Wall street history: Railroads and Rockefeller*. Investopedia. Retrieved March 23, 2023, from https://www.investopedia.com/financial-edge/0510/wall-
street-history-railroads-and-
rockefeller.aspx#:~:text=The%20railroads%20were%20among%2 0the,lines%20would%2 0follow%20the%20tracks.

Bell, L. (2021, November 29). *Evolution & History of Investing*. Ally: Do It Right. Retrieved March 23, 2023, from https://www.ally.com/do-it-right/investing/evolution-and-history-of- investing/

Beyoud, L. (2022, July 19). *Trading 'gamification' is huge concern, sec enforcement chief Gurbir Grewal says*. Bloomberg.com. Retrieved March 26, 2023, from https://www.bloomberg.com/news/articles/2022-07-19/ trading-gamification-concern-for- sec-enforcement-chief-says#xj4y7vzkg

Bonaparte, Y., & Cooper, R. (2010, November 17). *Rationalizing trading frequency and returns.*

National Bureau of Economic Research. Retrieved March 26, 2023, from https://www.nber.org/system/files/working_papers/ w16022/w16022.pdf

Boutté, C. (2022, June 4). *I lost over $3,000 by following bad investing advice on YouTube, but it taught me an important lesson.* Personal Finance. Retrieved March 26, 2023, from https://www.businessinsider.com/personal-finance/lost-money-following-bad-investing-advice-youtube-2022-6

Caporal, J. (2021, August 3). *Gen Z and millennial investors: Ranking the most used, trusted investing tools.* The Motley Fool. Retrieved March 23, 2023, from https://www.fool.com/research/gen-z-millennial-investors-tools/

Case, J. (2021, November 18). *Millenials and Gen Z are a growing force in investing. The market needs to catch up.* Fortune. Retrieved March 23, 2023, from https://fortune.com/2021/11/18/millennials-genz-investing-markets-wealth-transfer/

CFA Institute. (2018, October). *Uncertain futures: 7 myths about millennials and Investing – CFA Institute.* FINRA Investor Education Foundation. Retrieved March 23, 2023, from https://www.cfainstitute.org/-/media/documents/support/advocacy/1801081-insights-millennials-and-investing-booklet.ashx

Cook, A. (2022, March 29). *Every generation wants to retire comfortably.* MagnifyMoney. Retrieved March 25, 2023, from https://www.magnifymoney.com/news/investing-by-generation-survey/

Evans, J. R. (2022, June 7). *80% of gen Z investors took on debt to invest.* MagnifyMoney. Retrieved March 23, 2023, from https://www.magnifymoney.com/news/debt-to-invest/

Federal Reserve, Distribution of Household Wealth in the U.S. since 1989 (2022). Retrieved March 23, 2023, from https://www.federalreserve.gov/releases/z1/dataviz/dfa/distribute/chart/#quarter:126;series:Corporate%20equities%20and%20mut

ual%20fund%20shares;demographic:age;population:7;units:leve ls;range:1989.3,2021.1.

Hartmans, A. (2022, October 3). *If you bought EMAX crypto tokens when Kim Kardashian promoted them on Instagram, you'd have lost over 95% of your money by now.* Business Insider. Retrieved   March   30,   2023,   from https://www.businessinsider.com/kim-kardashian-   crypto-emax-ethereummax-sec-promotion-value-plummeted-2022-10

Hasso, T., Muller, D., Pelster, M., & Warkulat, S. (2021). Who participated in the GameStop frenzy? Evidence from brokerage accounts. *Finance Research Letters*, *43*, 1–16.

Hawkins, L. E., Versace, C., & Abssy, M. (2022, March 31). *Gen Z: What to know about the next generation of investors.* NASDAQ.   Retrieved   March   23,   2023,   from https://www.nasdaq.com/articles/gen-z%3A-what-to-know-about-the-next-generation-of- investors

How to protect your 401(k) from a stock market crash? Beagle. (n.d.). Retrieved March 24, 2023, from https://meetbeagle.com/ resources/post/how-to-protect-your-401-k-from-a-   stock-market-
crash#:~:text=While%20bonds%20don't%20return,from%20a%2 0stock%20market%20cra sh.

Kusumaningrum, T. M., Isbanah, Y., & Paramita, R. A. S. (2019). Factors affecting investment decisions: Studies on young investors. *International Journal of Academic Research in Accounting, Finance and Management Sciences*, *9*(3). https://doi.org/10.6007/ijarafms/v9- i3/6321

Library of Congress. (n.d.). *Wall street and the stock exchanges: Historical resources.* Stock Exchanges – Wall Street and the Stock Exchanges: Historical Resources – Research Guides at Library of Congress. Retrieved March 23, 2023, from

https://guides.loc.gov/wall-street-   history/
exchanges#:~:text=American%20Stock%20Exchange&text=Fou
nded%20by%20th

e%20National%20Association,trading%20for%20over%202%2C5
00%20securities.

Macheel, T. (2022, May 2). *Warren Buffett gives his most
expansive explanation for why he doesn't believe in bitcoin.*
CNBC. Retrieved March 25, 2023, from https://www.cnbc.com/
2022/04/30/warren-buffett-gives-his-most-expansive-
explanation-   for-why-he-doesnt-believe-in-
bitcoin.html#:~:text=Despite%20a%20shift%20in%20public,prod
uce%20anything%2C% E2%80%9D%20Buffett%20said.

Millennials, Gen Z and the Coming "Youth Boom" Economy.
Morgan Stanley. (2019, January 25). Retrieved March 24, 2023,
from https://www.morganstanley.com/ideas/millennial- gen-z-
economy.html

Mint, T. R. (2022, March 16). *Gen Z Investment Report.*
LinkedIn.   Retrieved   March   24,   2023,   from
https://www.linkedin.com/pulse/royal-mints-gen-z-
investment-report-the-royal-mint/

Morning Consult. (2022, March). *ETPS are empowering the
next generation of investors – NASDAQ.* NASDAQ. Retrieved
March   23,   2023,   from   https://www.nasdaq.com/docs/etps-
empowering-next-generation-of-investors

Newall, P. W., & Weiss-Cohen, L. (2022). The gamblification
of investing: How a new generation of investors is being born
to lose. *International Journal of Environmental Research and
Public   Health,   19*(9),   5391.   https://doi.org/10.3390/
ijerph19095391

Pepler, A. (Ed.). (2020, December). *Winter 2020 Better Money
Habits® Millennial Report.* Retrieved March 24, 2023, from

https://about.bankofamerica.com/content/dam/about/report-center/bmh/2020/2020-bmh- millennial-report.pdf

Sweney, M. (2022, October 3). *Kim Kardashian to pay $1.26m to settle crypto charges*. The Guardian. Retrieved March 30, 2023, from https://www.theguardian.com/lifeandstyle/2022/oct/03/kim-kardashian-settle-crypto- charges-instagram-post#:~:text=Kardashian%20asked%20her%20then%20220,into%20crypto%3F%3F%3F%3F%E2%80%9D&text=The%20post%2C%20which%20featured%20the,how%20to%20buy%20the%20tokens.

Ultra-Wealthy Risk Tolerance. (2014). *Spectrum High Net Worth Advisor Insights*, *10*(10), 2–3.

United States Department of Health and Human Services. (2021, May). *2020 Profile of Older Americans*. ACL administration for Community Living. Retrieved March 26, 2023, from https://acl.gov/sites/default/files/aging%20and%20Disability%20In%20America/2020Profi leolderamericans.final_.pdf

US Census Bureau. (2021, December 16). *Bicentennial edition: Historical statistics of the united states, colonial times to 1970*. Census.gov. Retrieved March 23, 2023, from https://www.census.gov/library/publications/1975/compendia/hist_stats_colonial- 1970.html

Vise, D. A. (1989, December 31). *The stock market from the roaring '80s to the sober '90s*. The Washington Post. Retrieved March 23, 2023, from https://www.washingtonpost.com/archive/business/1989/12/31/the-stock-market-from-the-

roaring-80s-to-the-sober-90s/
8f5858ec-18eb-4eb0-a822-3ba2bc2c0cdf/

Weller, C. E. (2002, April 10). *Learning lessons from the 1990s: Long-term growth prospects for the U.S.* Economic Policy Institute. Retrieved March 23, 2023, from https://www.epi.org/publication/webfeatures_viewpoints_l-t_growth_lessons/

Winkleman, C. S. A., Santamaria, C., & Winkleman, A. (2022, November 1). *The graphic truth: 50 Years of US inflation vs interest rates.* GZERO Media. Retrieved March 23, 2023, from https://www.gzeromedia.com/the-graphic-truth-50-years-of-us-inflation-vs-interest-rates

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6. Research Reflection by Kacey Tollefson

Kacey Tollefson

Faculty Mentor: Nathan Seegert (Department of Finance, University of Utah)

Participating in undergraduate research has enriched my educational experience at the University of Utah. Not only have I had the opportunity to work with excellent faculty mentors on projects but I've also been able to explore personal areas of interest with my own academic research. These experiences have allowed me to capitalize on my educational achievements at the University while contributing to my future field of work. I anticipate using the skillsets I have learned to create more informed decisions and practices throughout the rest of my life.

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SECTION II

School for Cultural and Social Transformation

7. An In-Depth Analysis of The Court System's Response to a Child's Psychological Well-Being in Domestic Violence Custody Hearing

Ximena Franco; Annie Fukushima; and Ronni Bateman

In a child custody hearing where domestic violence is present in the household, court appointed evaluators are tasked to investigate the nature of the alleged domestic abuse, the offender and victims, and the threat of ongoing violence after the separation. Preceding research outlines both the court's tendency to require joint custody and the psychological resources that are offered to the separating parents. If there exists the risk of domestic violence continuing or evolving after the separation, children who remain in joint custody situations are at risk of continued exposure to violence. The psychological burden that domestic violence may have on a child is of serious concern. Exposure to domestic abuse, in all its forms, negatively impacts the cognitive, social, and attachment development of children. Regarding hearing the victims' narratives and receiving psychological intervention, children remain to be an underrepresented demographic. An in-depth analysis of public court records and transcribed interviews with domestic abuse survivors were conducted to identify the existing tendency the courts may or may not have in taking the child's psychological well-being into consideration, and if any form of counseling or intervention was court mandated to protect those children.

Keywords: domestic violence, child custody, psychological intervention, joint custody

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8. **Research Reflection by Ximena Franco**

Ximena Franco

Faculty Mentor: Annie Isabel Fukushima (Ethnic Studies, University of Utah)

When beginning studying my major, I was surprised to learn how many demographics- across cultures, religions, political spheres, and communities- are generally left unprotected from criminal activity. One of those unprotected demographics that broke me was children. As I began doing personal research on how children become victims of criminal behavior, I realized that there remains many knowledge gaps. My research question aimed to address one of the knowledge gaps and analyze how the Utah court system responds to a child's psychological needs after witnessing/experiencing domestic violence. The concept is very difficult and there were moments where I need to step away and calm down before continuing. However, it has become more and more clear that even in the

justice system, children are not completely safe; their needs for stability and safety are not fully met. This remains a troubling thought for me and it begs the questions that if children are currently not safe in the system, where are they safe? And how can the system become a place where a child's safety is at the forefront of any decision making? Before my research, I was interested in achieving a legal career, either in the criminal or immigration fields. However, I now am considering pursuing a career in child welfare/advocacy. Whether it means I become a therapist for children, achieving a position of counselor/advisor for the local or state government, a lawyer specializing in child protection, or becoming a foster parent, I aim to work in some capacity that may be able to provide safety and resources to children who experience danger in their lives.

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9. Migratory Monsters

Sandra Del Rio Madrigal

Faculty Mentor: Annie Isabel Fukushima (Ethnic Studies, University of Utah)

In collaboration with online space Migratory Times, I developed a creative research project to work on my novella, Silk Skin, over the course of Fall semester 2021. The narrative follows two Mexican-Americans—a mother named Lorena and her daughter Adela—who welcome two migrants from Tijuana to their home. Adela's kinship with the Tijuanenses challenge previous relationships as memories resurface of a monstrous figure who may be the one making mysterious noises at night.

To develop this creative project, in collaboration with Migratory Times, the Center for Arts Design & Social Research, and my faculty mentor (Dr. Annie Isabel Fukushima), we organized a series entitled Migratory Monsters. The series fostered research through conversations with artists and scholars Kakyoung Lee, Rebecca Close, Dr. Angela Marie Smith, and Diana Tran. Here I fostered my own creative

research project grappling with diaspora and representations of Othered identities in the context of horror. Horror exposes institutional oppression. How do we further employ horror tropes to liberate transnational identities? How do we abandon normative, untroublesome narratives?

We held the events "Visions of Monstrosity," and "The Body and Horror." Speakers discussed corporeality, erasure, and the complexity of liberating oneself from colonial constraints. The piece's title, Silk Skin, emphasizes the creation of self through unconsumed subjects. The title also references a vital scene in the story which apprehends complacency in Utah by stripping an antagonist's sense of security and literal skin.

The excerpt below involves the introduction of the story's monster figure, Samara titled "Visions of Samara."

She came loose-limbed, bones bent in all the wrong places. She didn't breathe, didn't require air, didn't gasp for air as one should have. From my room, all I saw was a rag, a burlap sack perhaps, slumping its way across the small yard.

I had first assumed a neighbor's remote-control racecar drove the rag beneath. When she stood up, though, her shape revealed a strange form, an imitation of what I looked like. Where I stood ramrod straight, she curled against herself, and where I was tense, she was soft. Where I was clean and pristine, she was dirty, stained, and bloody.

When she took two steps forward from the edge of the property, her feet flapped, like oversized socks pushed out from underneath her toes. Her long hands almost held her head from the waist as she trembled. Then, she extended upwards to face me as I faced her from behind my window, yet her back arched backwards exaggeratively. I feared she would fall from the unbalance, but she gracefully continued her steps towards the backdoor. Her

hands dragged backwards on the dry grass behind her as she advanced.

When I waved, she waved. When I craned my neck, so did she. When I ran to the door to welcome the disfigure, she looked inside my home before coming in.

The burlap that coated her body was rough to unstitch.

I flustered as the silk knotted beneath the needle's steady beat—again. The light layers of fabric were difficult to piece together as a novice. Despite my careful effort, the sewing machine ate the brown silk and chewed until the bobbin thread ripped. I sighed and pulled hard until the fabric came loose with a small snapping following it through. The loose threads looked like small body hairs coming out from the seams. It didn't look unnatural. It added texture.

After cutting the excessively long thread from where the stitching began, the piece almost looked completed. To me, at least. Doña Esme didn't think it looked like anything.

"Mija, look here," she pointed at where the fabric had been chewed, "what kind of needle are you sewing with?"

I shrugged. I had no idea there was difference between certain needles.

"Mira, silk is slippery, verdad? You've probably noticed that it slips, and that it's thin. That's because silk is a very special kind of fabric. You've chosen it for a reason, no? The fact that you are using it means you have good taste. But you must be gentle with it. The needle you are using is thick, and it pushes it all the way down into the machine, down to the bobbin. And then it won't come up. Use a thin needle, like this one right here. You have all the needles you need in the pouch next to the machine. Let's fix it up so that you can so your, uh, your ropita right here."

She taught me to change the needle, along with the foot. After, she left me to complete the piece.

I had taken Samara's measurements, had wrapped the tape around each angle and curve of her body until I knew how I could replace the burlap skin. The burlap had been dirty, had been stained by countless shades of browns, greens, reds, and yellows. What was contained on the inside was worse—her muscles and bones had consisted of shards of glass, sticks, dirt, grass, along with gooey gunk. When I had asked her what the unfamiliar materials were, Samara only shook her head and waited for me to move on.

But I had seen that gunk at the markets where my mother stood in line to ask the butcher for specific quantities of meat. I had seen that gunk when she bought chamorro de res, carne ranchera, diezmillo, and trozo. I had seen the way this gunk oozed with blood as our sharpest knife came down to pull chunks off a bone. How all that meat could dry and become brown, sometimes green if we forgot to make a stew before it rotted.

Instead of saying, so, though, I went through her contents, pulling sticks through thick, long paper straws, and replacing the gunk with fabric scraps, or old buttons and beads. She molded herself with a light attitude, and asked me to discard the burlap sack (which now contained the gunk we had taken out.)

I promised her I would bring her a prettier fabric for her to wear as a new skin. She asked for one as pretty as mine. Then, she needed only to shimmy into the silk skin I had made for her.

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10. Research Reflection by Kathryn Howard

Kathryn Howard

Faculty Mentor: Lisa Diamond (Psychology and Gender Studies, University of Utah)

This research has inspired me to pursue topics I am passionate about that have the potential to help people. It has also prepared me for applying to grad school and further my education.

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11.   **Toward a New Future: An Oral History Study Examining K-12 Ethnic Studies Education in Utah**

Haley Tetzlaff

Faculty Mentor: Thomas Michael Swensen (Ethnic Studies, University of Utah)

Abstract

In May of 2022 Utah passed bill SB244 stating the requirement of Ethnic Studies education in K-12 institutions. Because of this, in the spring of 2023 we conducted a series of oral history interviews with a current student, an alumna, and two high school Ethnic Studies teachers to investigate the impact it K-12 Ethnic Studies has in Utah. With this mix we were able to

capture a broader range of experiences. Prior to this study, when looking into K-12 Ethnic Studies research conducted in Utah, there was nothing to be found. The goal of this research was to fill that gap and find out the importance of K-12 Ethnic Studies education in Utah by highlighting the voices of those with firsthand experience through collecting their oral histories, in hopes of bringing to light how Ethnic Studies can/will be beneficial to Utah public education. This research was fueled by 2 questions. (1) How has K-12 Ethnic Studies education impacted people with firsthand experience in Utah thus far, and (2) How will bill SB244 be beneficial to the Utah state public education system? These 2 questions paired with the bolstering of student and teacher voices has molded this research. The findings suggest that the participants in this study had a positive and world expanding experience with K-12 Ethnic Studies in Utah. Specifically, K-12 Ethnic Studies has helped participants expand relationships, empathy, and solidarity. As well as, help expand self-reflection and appreciation, cultural value, and critical thinking, discussion, and examination skills. The findings show, if done correctly, SB244 could be very beneficial to Utah public education.

Bibliography

Brown, Keffrelyn D., and Anthony L. Brown. 'Silenced Memories: An Examination of the Sociocultural Knowledge on Race and Racial Violence in Official School Curriculum'. Equity & Excellence in Education 43, no. 2 (7 May 2010): 139–54. https://doi.org/10.1080/10665681003719590.

Chapman, Thandeka K., Makeba Jones, Ramon Stephens, Dolores Lopez, Kirk D. Rogers, and James Crawford. 'A Necessary Pairing: Using Academic

Outcomes and Critical Consciousness to Dismantle Curriculum as the Property of Whiteness in K-12 Ethnic Studies'. Equity & Excellence in Education 53, no. 4 (1 October 2020): 569–82. https://doi.org/10.1080/10665684.2020.1791767.

Curtin, Joseph A. "Utah's Growing Opportunity Gap Final Updated Template – Ushe." Utah System of Higher Education, August 2019. https://ushe.edu/wp-content/uploads/2019/07/Utahs-Growing-Opportunity-Gap-Final_updated-template.pdf.

Dee, Thomas S., and Emily K. Penner. 'The Causal Effects of Cultural Relevance: Evidence From an Ethnic Studies Curriculum'. American Educational Research Journal 54, no. 1 (February 2017): 127–66. https://doi.org/10.3102/0002831216677002.

Ginwright, Shawn, and Julio Cammarota. 'New Terrain in Youth Development: The Promise of a Social Justice Approach'. Social Justice 29, no. 4 (90) (2002): 82–95. https://www.jstor.org/stable/29768150.

Harris, Cheryl I. 'Whiteness as Property'. Harvard Law Review 106, no. 8 (June 1993): 1707. https://doi.org/10.2307/1341787.

Kohli, Rita, Marcos Pizarro, and Arturo Nevárez. 'The "New Racism" of K–12 Schools: Centering Critical Research on Racism'. Review of Research in Education 41, no. 1 (March 2017): 182–202. https://doi.org/10.3102/0091732X16686949.

Kohli, Rita, and Daniel G. Solórzano. 'Teachers, Please Learn Our Names!: Racial Microaggressions

and the K-12 Classroom'. Race Ethnicity and Education 15, no. 4 (September 2012): 441–62. https://doi.org/10.1080/13613324.2012.674026.

Mikulyuk, Ashley B., and Jomills H. Braddock. 'K-12 School Diversity and Social Cohesion: Evidence in Support of a Compelling State Interest'. Education and Urban Society 50, no. 1 (January 2018): 5–37. https://doi.org/10.1177/0013124516678045.

Pawel, Miriam. "Ethnic Studies in California." Education next 21, no. 3 (2021): Education Next, 2021, Vol.21 (3).

Oto, Ryan, Abby Rombalski, and Justin Grinage. 'The Role of Racial Literacy in US K-12 Education Research: A Review of the Literature'. Race Ethnicity and Education 26, no. 1 (2 January 2023): 94–111. https://doi.org/10.1080/13613324.2022.2047635.

Tetzlaff, Haley. 'Self Respect and Me' (2019): 1–4. https://docs.google.com/document/d/1irQFaW_PVVi_nT80ZQqLIyFQqPkEi7x36_trZhEYONc/edit?usp=sharing

Tintiangco-Cubales, Allyson, Rita Kohli, Jocyl Sacramento, Nick Henning, Ruchi Agarwal-Rangnath, and Christine Sleeter. 'Toward an Ethnic Studies Pedagogy: Implications for K-12 Schools from the Research'. The Urban Review47, no. 1 (March 2015): 104–25. https://doi.org/10.1007/s11256-014-0280-y.

Utah State Legislature Ethnic Studies

Commission, 2022. https://le.utah.gov/av/committeeArchive.jsp?mtgID=18383.

Vue, Rican. 'Ethnic Studies as Interest Divergence? Countering Racial Neoliberal Politics and Envisioning a Beloved Community with Racial Literacy'. Race Ethnicity and Education 26, no. 1 (2 January 2023): 54–72. https://doi.org/10.1080/13613324.2021.1890562.

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12. **Research Reflection by Haley Tetzlaff**

Haley Tetzlaff

My undergraduate research helped me see myself as capable of doing research. It also helped me see the potential that research has. Research can be anything you want. It can be creative, fun, and impactful all at the same time. My project has impacted me because it made me realize that I want to become a high school Ethnic Studies teacher one day.

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SECTION III

College of Education

13.   **What Should the Main Roles of Public Elementary Education Be in the United States? An Exploratory Study Based on Survey Responses of Teachers During the COVID-19 Pandemic**

Tessa Cahoon

Faculty Mentor: Mary Burbank (Education, Culture & Society)

Introduction

Education is an important value in American society. Public schools are expected to fill many spoken and unspoken roles in our society. These roles range from teaching academics and preparing students for the workforce to teaching English, providing food for low-income students, keeping students safe, and meeting the individual needs of all students, including those with disabilities (Spring 2018).

During the 2020-21 school year, COVID-19 put a significant strain on the public education system. Teachers were forced to take on more responsibilities, all within a context of limited resources and extra challenges (both personal and public). They also had to adapt quickly to new conditions and expectations as well as re-evaluate their teaching priorities (Arnett, 2021). Thus, this was an opportune time to revisit what we expect from our schools and to assess how realistic our expectations are, particularly since education continually faces new challenges and changing needs and demographics.

Methods

An online survey was sent out to seven elementary schools in City View District (a pseudonym). Data were gathered in accordance with Institutional Review Board (IRB) approval. Teachers were asked about how effective they were at meeting a list of seven expectations based on the 2013 Interstate New Teacher Assessment and Support Consortium

(InTASC) and the Utah Effective Teaching Standards (UETS). Additionally, teachers were asked how COVID-19 affected their ability to meet these expectations and how their school and community prioritized these expectations. The survey's open-ended questions were analyzed quantitatively by grouping responses into themes and categories, and the survey's close-ended questions were analyzed using descriptive statistics.

Results

- During an average school year, teachers' top four priorities were building relationships with students, meeting the individual needs of all students, teaching the Utah Core Standards and providing culturally responsive instruction. Teacher priorities remained consistent during the 2020-21 COVID-19 year, with the unsurprising exception that physical safety tied with building relationships with students for the top priority.

- Teachers' ability to meet expectations matched their top four priorities, with one big exception: meeting the individual needs of all students. Only 68% of teachers felt they could meet this expectation during an average year; during the 2020-21 school year, this dropped to 45%.

- The COVID-19 pandemic made it more challenging for teachers to meet the majority of the expectations; however, the most marked declines were meeting

the Utah Core Standards and adapting/updating teaching instruction.

- 76% of respondents felt that teaching expectations were realistic in an average school year, but this dropped steeply to 28% during the 2020-21 school year. However, even during an average year, 24% of teachers felt expectations were unrealistic.

- Respondents reported needing more support in meeting the academic and mental health needs of students and in providing students with differentiated instruction. Respondents also reported needing better mental health support and better financial compensation for themselves.

Conclusion

The COVID-19 pandemic undeniably made public education more challenging during the 2020-21 school year; however, teachers were already struggling to meet all of the expectations placed upon them before the pandemic. As a nation, we need to have important conversations about what we really value and expect from our teachers. Teachers need more support and resources in order to effectively fulfill their teaching responsibilities, particularly as the needs of students become more diverse and teaching conditions continue to be complex and challenging.

References

Arnett, T. (2021, January). Breaking the mold: How a global pandemic unlocks innovation in K-12 instruction. Clayton Christensen Institute for Disruptive Innovation.

https://www.christenseninstitute.org/wp-content/uploads/2021/01/BL-Survey-1.07.21.pdf

Council of Chief State School Officers. (2013, April). InTASC Model Core Teaching Standards and Learning Progressions for Teachers 1.0. https://ccsso.org/sites/default/files/2017-12/2013_INTASC_Learning_Progressions_for_Teachers.pdf

Spring, J. H. (2018). American education (18th ed.). Routledge.

Dupree, K. M., & Hill, K. (2013). Utah Effective Teaching Standards and Indicators. https://www.schools.utah.gov/file/7313cfe5-5e68-41ef-9de4-03e5a9d395d8

Figure 1. Teachers' Prioritization of Expectations During an Average Year vs. the 2020–21 COVID–19
School Year

Teachers' Prioritization of Expectations During an Average Year vs. the 2020-21 COVID-19 School Year
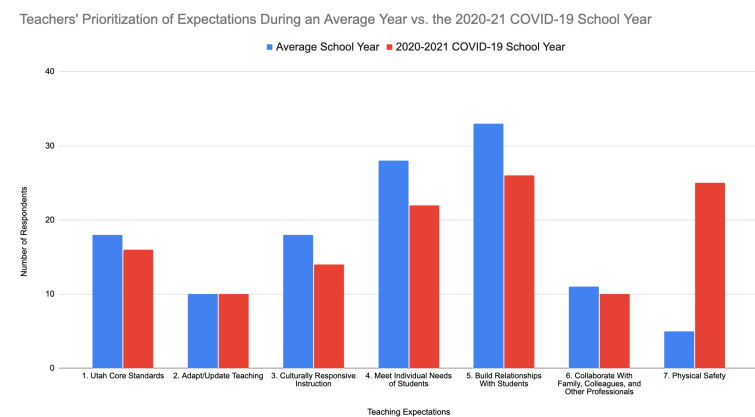
Figure 2. Extent Expectations Were Met During an Average School Year

Extent Expectations Were Met During an Average School Year
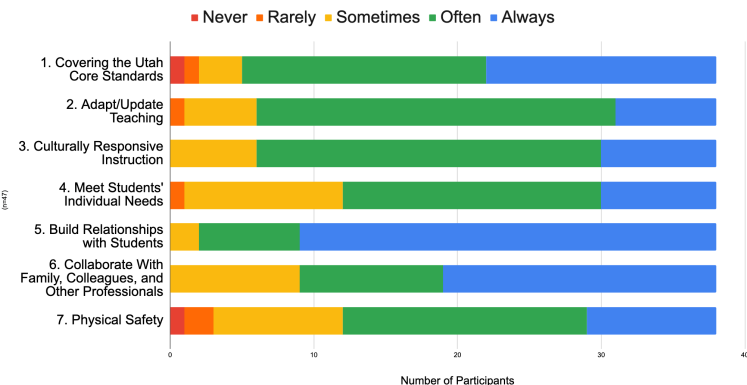
Figure 3. Extent Expectations Were Met During the 2020–21 COVID–19 School Year

Extent Expectations Were Met During the 2020-21 COVID-19 School Year
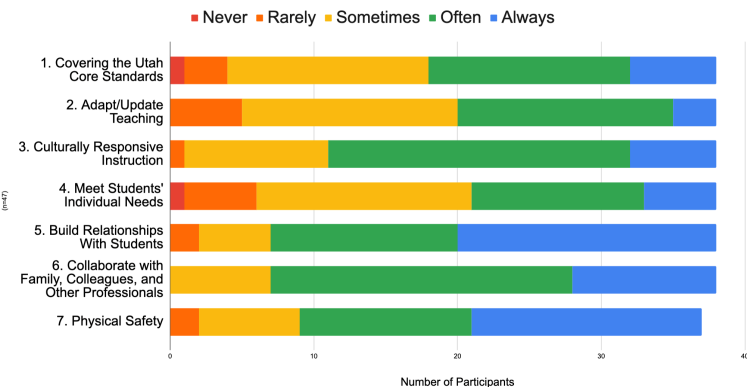
Figure 4. Are These Expectations Realistic?

Are These Expectations Realistic?

2020-21 COVID-19 School Year   Average School Year
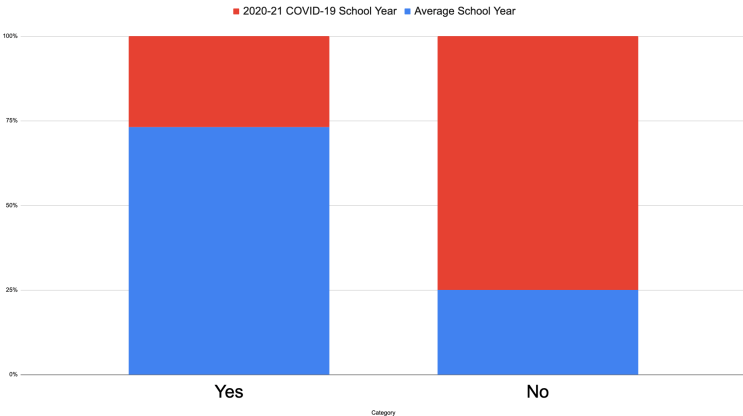
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14.   Middle School Student-Generated Ideas About Math: Definitions and Utility

Alyssa Lee

Faculty Mentor: Tracy Dobie (Educational Psychology, University of Utah)

Abstract

This study investigates student-generated ideas about mathematics, focusing specifically on students' definitions of math and their notions of math's utility. The study is designed to explore these concepts concurrently through two research questions: (1) How do middle school students define mathematics? (2) How do those definitions connect with or relate to their ideas of math utility? Student-generated ideas are central to this exploration because they offer more insight

into what students are learning and how they are motivated to learn (Canning & Harackiewicz, 2015; Eccles & Wigfield, 2002; Johansson & Sumpter, 2020). I focus on middle- school students as they are at a critical period in their educational careers and perceptions of math (Priniski & Thoman, 2020). Students participated in semi-structured interviews, and select questions were chosen for analysis. Findings reveal that most students primarily thought about math content over math processes when offering ideas about defining math. Additionally, there were connections between which areas of math were mentioned in connection with which domains of utility. Among content areas defined by the National Council for Teachers of Mathematics (NCTM), *Numbers & Operations* and *Measurement* were most frequently connected to *Daily Activities*. Among math processes defined by the NCTM, *Problem Solving* and *Connections* were most frequently connected to *Future Job/Career*. However, there was not a connection between the breadth of a student's math definitions and the breadth of their notions of math's utility. These findings have implications for what kinds of instructional decisions teachers make in their classrooms, and how they try to connect math content and processes to their students' personal lives.

IntroductionMany educators like to project the idea that "math is everywhere" to ingrain a sense of its utility in students. Students frequently echoed this idea when asked questions about the usefulness of math (Dobie, 2019a). However, when students are given the opportunity to discuss mathematics, they experience difficulties when making these connections beyond the classroom (McDonough & Sullivan, 2014) or when thoughtfully and meaningfully applying a math

concept to a real-world context (Metzger et al., 2019). Thus, students are not finding examples of math's utility "everywhere." Students mostly refer to money and future jobs when thinking about math utility (Dobie, 2019b). Existing literature has shown that when students think of math as useful, they are likely to have improved academic achievement and higher personal interest in math, and this can be especially impactful and beneficial for students with lower mathematics performance (Hulleman & Harackiewicz, 2009). However, it is important that researchers understand students' ideas about utility rather than just asking if students think math is useful (Dobie, 2019b; McDonough & Sullivan, 2015). This difference is important because when students are only given the opportunity to think of usefulness in terms of teachers' examples, it can be detrimental for students who have a low sense of personal math competence and who have not personally connected with those notions of utility (Hulleman & Harackiewicz, 2009). Additionally, this difference between students' own ideas of utility and the generalized belief that math is useful is important when considering the scope of ideas represented in research on math utility. The representation of more diverse notions of math utility can lead to more equitable math classrooms (Dobie, 2019b). Thus, there is a need to consider what students' perspectives may add to knowledge of effective math instruction.

While past studies point to limited perceptions of math utility, other research demonstrates that students may also have limited definitions of math. In one study, student- generated definitions of mathematics from students in grades one through four were limited to numbers, counting, and basic

operations, which left out many math domains including algebra, geometry, measurement, and problem solving (Metzger et al., 2019). Other research with students in grades two through eight in elementary and middle school demonstrated that students simply struggle when tasked with defining math (McDonough & Sullivan, 2015).

Emotional responses are tied to conceptions about math, and the emotional experiences of math are shown to become more negative from year to year starting as early as elementary school from grades two to five (Johansson & Sumpter, 2010). Students express more frustration as the math becomes more complex. As a result, elementary students may disengage from expanding their scope of what math is. However, this disengagement is not the choice of students; it is the result of math instruction that teaches them to understand math as something that exists in the classroom, in a book, and at a desk (Johansson & Sumpter, 2010). Interview responses from students showed that students do not associate math learned in school with daily math encounters beyond the classroom (Metzger, et al., 2019). Because math instruction is often abstract and not explicitly tied to these daily encounters, students are not offered channels to make meaningful math connections that would expand their scope and definition of mathematics. Understanding student definitions of math is important for teachers to be responsive

to what students already know for effective instruction including making more explicit math connections to real world contexts (McDonough & Sullivan, 2015; Metzger, et al., 2019; Moll et al., 1992).

Both ideas of math utility and definition are important for effective instruction that maintains student motivation. Given that one's way of defining math is likely to play a role in where one sees math in the world, there is a need to study these concepts concurrently. Studying these concepts through middle school student ideas provides insight into a critical period in students' formation of their math identities and ideas (Priniski & Thoman, 2020). The current study explores two research questions: (1) How do middle school students define mathematics (2) How do those definitions connect with or relate to their ideas of math utility? Exploring these questions may have implications for how teachers make instructional decisions in math.

Literature Review

The research questions rely on existing literature about students and their ideas about mathematics. First, I consider research about how students construct their own definitions of math and how math is defined by instructional standards. Then, I consider Expectancy-Value Theory to understand how notions of utility are related to students'

learning motivation. Next, we discuss the importance of using student-generated ideas.

Definitions of Math

To explore how students define mathematics, it is important to consider what is involved in the formation of their conceptions. Johansson and Sumpter (2020) define "conception'' in relation to students: "…an abstract or general idea that may have both affective and cognitive dimensions inferred or derived from specific instances. Hence, students' conceptions consist of their belief systems, values, and attitudes reflecting their experiences" (Johansson & Sumpter, 2020, p. 78). Establishing this definition allows an open-ended exploration of student ideas as to not limit student-generated ideas, which are essential to the research purpose.

There are multiple aspects to consider with this definition. First, a conception is general in the sense that it is not comprehensive or absolute, and it is dynamic. Second, student ideas or conceptions will involve affective characteristics. These include how they feel about math, what they think math is for, and what they think math looks like. Conceptions also involve cognitive dimensions where students might give more explicit and tangible answers of what math is through examples. Considering these components that make up students' ideas, teachers and researchers should be mindful that student

responses are limited to their personal experiences and perspectives.

Because student conceptions of math are dependent on their own experiences, it is important to consider existing research that focuses on how students imagine themselves in relation to math. Johansson and Sumpter (2020) explored this element in a qualitative analysis by asking students to draw a picture of themselves when they are engaging with math. Second and fifth grade student illustrations led to the same conclusion where students imagined themselves alone and working in a book; this was their image of what it means to do math (Johnasson & Sumpter, 2020). The researchers compared these findings to observations of the students in class. Those observations revealed that these depictions were in line with the kinds of math activities that happened in their classrooms, where students were observed working alone or in small groups and with their textbooks. While this encourages teachers to re-evaluate their instructional strategies in math, it also demonstrates that students' ideas are clearly reflective of their lived experiences in line with Johansson and Sumpter's definition of "conception" related to math ideas.

In addition to allowing student responses to incorporate their personal experiences, a thorough analysis of definitions requires mechanisms for coding student ideas to understand them in

collection. Metzger and others (2019) provide ways to decipher students' math definitions using standards from the National Council of Teachers of Mathematics (NCTM) (NCTM, 2000). Using standards that guide teachers' math instruction, they divide students' math knowledge into content and processes: "Content includes number and operations, algebra, geometry, measurement, and data analysis and probability. Processes include problem solving, reasoning and proof, communication, connections, and representations" (Metzger et al, 2019, p. 432; NCTM, 2000). By presenting a way to dissect student definitions, these authors offer a way to evaluate and compare student responses.

This organization of math ideas into either content or process is central to Metzger's exploration of student knowledge of math across grade levels and how students conceptualize its usefulness (Metzger et al., 2019). As part of their method, students were interviewed and asked several questions about math. One key question was: "What is math?" (Metzger et al, 2019).

Based on their coding of student responses, a vast majority (91.9%) of students from grades one through four talked about math through its content. In contrast, only 12.1% of responses involved math processes, demonstrating a limited scope in thinking about math. Even when looking at those 91.9% of responses that talked about math

content, most of those responses primarily focused on *Numbers & Operations* (89.9% of all responses). Other areas of math content including *Algebra*, *Geometry*, and *Measurement* were all mentioned by fewer than 10% of students (*Algebra* was discussed by 3.0% of students, *Geometry* was discussed by 8.1% of students, and *Measurement* was discussed by 7.1% of students) (Metzger et al., 2019). These results indicate that students have very limited definitions of math when compared to standards of math instruction as defined by the NCTM. Metzger suggests that this may be because students' engagement with mathematics beyond the classroom is not explicitly connected to math, indicating a potential connection between students' ideas of definitions and utility (Metzger et al., 2019).

Expectancy-Value Theory and Utility Value

Theories of learning and motivation in educational psychology are useful for teachers to increase students' academic performance and engagement. Modern Expectancy-Value Theory (EVT) is a comprehensive and research-based model to consider the relationship between multiple variables related to student motivation. Some of those variables include students' perceptions of their own competencies, perceptions of the difficulty of various tasks, and personal goals. Through considering these complex variables together, Eccles and Wigfield (2002) provide a guide for educators and researchers in understanding

students' motivations and how to tap into their potential.

Eccles and Wigfield dive into student expectations of success, as well as their beliefs about their own abilities, which are tied to their motivation to participate in class. When students believe they can be successful and that they are capable, it is easier to justify expended effort in learning. However, if they conclude that they will not be successful or are not capable, students are less likely to engage (Eccles & Wigfield, 2002). However, it is not enough for students to believe they will be successful; they must also have a purpose to attempt achievement. Thus, the theory also includes task-values, which are broken into four components: attainment value, intrinsic value, utility value, and cost (Eccles & Wigfield, 2002). Attainment value is related to personal importance and identity-building. This may include a sense of pride by being successful at a given task or acquiring prestige. Intrinsic value is the enjoyment brought on by completing the task itself and the inherent value of the task. Utility value is the perception of how useful engaging in a given task will be for future goals such as career planning. Finally, cost considers the associated negative aspects of a task, such as loss of time or energy, or potential trade-offs. Utility value is a worthwhile component to investigate further because of its malleability.

Within science and mathematics instruction, a number of studies show that utility-value interventions have positive effects on students' academic outcomes. Hulleman and Harackiewicz (2009) demonstrated that when students are tasked with creating personal connections with the learning in their science courses, their interest and grades increased, and the effect size was greater for those who had low expectations of success. Canning and Harackiewicz (2015) reinforced those findings through their study on mathematics education, and they also emphasized that students are active in making these connections to their personal lives rather than just accepting the connections that their instructors have offered. Brown et al. (2015) explored how interventions focused on expanded notions of utility, such as including communal utility with existing ideas of self-oriented utility, led to both short-term and long-term increases in learning motivation in biomedical sciences. While these utility-value interventions demonstrated increased academic performance and motivation, another study found that a high perception of utility value in science and mathematics was a significant factor contributing to students' self-selection of those courses in the earlier stages of students' education (Caspi et al., 2019). Thus, a student's perception of math's utility value greatly influences their access to and selection for future math learning.

Student-Generated Utility

Utility value interventions that directly communicate the usefulness of math are not effective in improving academic performance for all students (Canning & Harackiewicz, 2015). In Canning and Harackiewicz's studies, they compared the effect of directly communicated utility math interventions and self-generated utility math interventions on students. Forms of directly communicated utility value come from sources like parents or teachers. In their design, participants were randomly assigned to one of two utility value interventions. They first had students participate in a self-generated utility value intervention where they generated examples of math's utility related to their own lives. The second had students participate in a directly communicated utility value intervention where students listened to an instructional presentation (Canning & Harackiewicz, 2015). Their findings reveal that directly communicated ideas of usefulness promoted performance for students with higher confidence in their math abilities, but they were detrimental to the performance of students who lacked confidence in math. In contrast, self-generated ideas of usefulness promoted math performance for all students, and this intervention was even more impactful for students with lower levels of confidence in their math abilities (Canning & Harackiewicz, 2015).

Student-generated ideas of utility allow students to imagine themselves as the users of math concepts. This research does not suggest that all directly communicated ideas of math utility value should be removed from the classroom. In the related studies, students' perceived utility value of math increased in settings where direct communication and student-generated ideas of utility value happened concurrently (Canning & Harackiewicz, 2015). They suggest this may be because when done together, students have the opportunity to put directly communicated ideas about usefulness in their own words. Thus, direct communication of utility value in instruction is still valuable. But Dobie (2019a) points out the potential harm of emphasizing the usefulness of content when students do not feel that they are capable of using it. Remembering EVT, students will not feel motivated to engage in a task if they do not feel they can expect success or if they perceive that they are not capable.

Incorporating student-generated ideas of math expands notions of what could be useful beyond what instructors are currently offering through direct communication and direct teaching. Dobie (2019a) asserts that math instruction should be designed for student populations beyond the dominant cultural group by discussing a previously ignored way to think about utility. She highlights the need to attend to models of the self (Grossmann

& Varnum 2011; Stephens et al. 2007) and discusses the differences between independent and interdependent conceptions of usefulness. Independent conceptions of usefulness focus on individual performance while interdependent conceptions of usefulness consider connectedness amongst individuals. Math instruction often focuses solely on independent conceptions of usefulness, but Dobie (2019a) suggests that some populations may benefit from expanded notions of utility that consider both perspectives. Other literature discusses similar ideas through agentic utility value which focuses on self-oriented usefulness and communal utility value which focuses on usefulness to help others (Brown et al., 2015). The effects of expanded notions of utility that include both self- oriented and communal utility values benefit all students in the classroom (Brown et al., 2015). Through acknowledging groups not previously considered, novel ideas emerge with serious potential to elevate student learning motivation. These expanded notions of utility are essential to consider when analyzing student-generated ideas about utility in math.

Approach to the Research

The current study focuses on understanding student-generated math definitions and notions of math utility concurrently. This is because there may be a correlative relationship between students' definitions and their sense of its utility. Metzger

and her colleagues (2019) show that students had a narrow scope of mathematics within their study on student conceptions of math utility. This research aligns with Johansson and Sumpter's (2010) study about how students think of math as something that occurs at a desk in class. This reinforces Metzger's (2019) findings where students shared math ideas demonstrating math as content where something is learned in school, and not a process or something they engage with or do as an action. To improve student motivation through utility value, existing research demonstrates that students must envision themselves as actors and users of math through generating their own ideas of its usefulness (Canning & Harackiewicz, 2015). Expanding their definitions of math to include processes and expanding their notions of its utility are thus related and should be studied concurrently.

The current study focuses on middle school students and their ideas about math because this age group is specifically important to consider as they are in a critical period regarding their relationship with math learning. During these years, in which there is greater emphasis on math and science learning, students display significant declines in learning motivation and begin to develop deeper personal identities as individuals and as learners (Dweck & Master, 2009; Eccles, 2009). Priniski and Thoman (2020) highlight that in the broader school structure of the United States,

middle school years are also the first time that students have autonomy in course selection and are more subject to being put on different course tracks. Thus, this time is indispensable in setting up the future of their opportunities for math education. With all these factors together, middle school students are an excellent group of sample participants for on-going research.

Methods

Data were collected through interviews with 8th-grade students attending an urban charter school in the Mountain West. These interviews were conducted between April and May of 2022. Interviews ranged from 26 to 45 minutes (mean = 35.7 minutes) and included questions focusing on students' ideas about mathematics and its usefulness. Student definitions of math were coded using the content and process standards from the National Council of Teachers of Mathematics similar to the coding in Metzger's study (Metzger et al., 2019; NCTM, 2023). Student conceptions of utility were coded using categories from Dobie's previous research (2019b) on student perspectives of math utility. The relationship between definition and utility was further explored by looking at the most common utility connections made with each math definition category.

Participants

Participants were identified for this study through a cooperating teacher. Students who

volunteered to participate received a $10 gift card incentive. Each of the 10 participants were in 8th-grade and aged between 13 and 14 years old (mean age = 13.4 years). Six students described their gender as male, 3 as gender non-conforming, and 1 as female. Students were asked to describe their race or ethnicity using as many identifications as they felt appropriate. Of the 10 participants, 40% identified as white, 40% identified as Hispanic or Latino, and 20% reported as both African American and Hispanic or Latino. In a post-interview survey, students were asked to answer a few questions about their own competencies in math on a scale from 1 to 7. The first of these questions asked: "How good at math do you think you are?" with 1 being "Not good at all" and 7 being "Very good." The mean response from participants was 4.8 (ranging from 3 to 6). Second, the students were asked: "If you were to rank all the students in your math class from the worst to the best in math, where would you put yourself?" with 1 being "The worst" and 7 being "The best." The mean response from participants was 4.5 (ranging from 3 to 6). Third, the students were asked: "How well do you think you will do in math this year?" with 1 being "Not well at all" and 7 being "Very well." The mean response from participants was 4.5 (ranging from 4 to 6).

Interviews

Interviews were semi-structured and largely guided by predetermined questions, but follow-up

questions were integral to the interview process. For the present study, select questions were chosen from the interviews to focus on the research questions.

Key questions used to analyze responses for student-generated definitions came from the early portions of the interview, and students were asked: "Imagine that a new kid moved to your neighborhood and that kid has never taken a math class before or even heard the word 'math'. Can you pretend I'm that new kid and tell me, what is math?" Some follow-up questions asked for more explanation or clarification. To expand their thinking on this question, students were also asked: "When I say the word 'math', what words or pictures come to mind?" The key question used to analyze and code responses related to math definitions differs from prior research. In Metzger's study, they asked: "What is math?" (Metzger et al., 2019). This difference in framing of the question was part of the design because of the difficulties students experienced in defining mathematics in prior research (McDonough & Sullivan, 2014). Thus, the question's framing is intended to offer context for students. It is intended to create better access to understanding what the question is asking and to create a launching point for students to craft their answers.

Key questions used to analyze responses for student-generated notions of math utility were

pulled from the beginning of the interview and from the later portions of the interview. One key question about utility from the beginning of the interview asked: "When I was a teacher, some of my students would say math is useful, and some of them would ask when we're ever going to use math and say it's not useful. What do you think? Who do you agree with?" Some common follow-up questions include: "Why do you think math is useful/not useful?", "Why do you think some of my students said math isn't useful?", and "If someone disagrees with you, how could you try to prove to them that math is (or isn't) useful?" The interviewers offered their own experiences through this question to encourage students to be more honest in their own responses when thinking about whether math is useful, so they do not say only what they think the interviewer wanted to hear. Additionally, this framing of the question asked students to explain why math is useful rather than just offer examples. While their answers may have included examples, it was focused on answering why math is useful rather than just how it is useful. This allowed more alignment with the coding scheme for math utility whereas examples of math being useful may have led them to continue answering how they would define math and what they think math includes.

Additional questions were selected for analysis from the end of the interview. The first of these

questions asked students, "Can you think of any people in your life who use math regularly?" This question is aimed to have students think about where they see math used and to frame it through their own experiences rather than just offer directly communicated forms of utility they may have received from teachers or others. This question required the notion of utility to come from the students' own observations. Similarly, the students were asked, "Do you use math regularly in your life?" to learn more about their personal experiences and conceptions of math. Follow-up questions were asked about when they use math, how they use math, and what kinds of math they use.

Next, students were asked, "Do you have a job or career in mind that you want to pursue in the future?" and if students responded with an example job or career, they were asked, "Do you think you will need to know math to get that job or to do that kind of work?" They were asked about how these related to math, and these questions allowed students to offer notions of utility that connected to their futures and not just their current experiences and daily routines. This connection to their future is essential to fully analyzing their sense of math's utility as it is a key component of utility value in EVT (Eccles & Wigfield, 2002).

Finally, students were asked, "Do you have any other kinds of goals for your future, or things that

are really important to you in your life?" and if they responded with examples, then they were asked: "Do you imagine math being helpful with those in any way? Why or why not?" It was important to not just ask about one potential area where they may use math in the future. For example, it would have limited the analysis if students were only asked about math in relation to their future career as this does not reflect diverse notions of utility (Dobie,

2019b). The end of the interview was left open for students to share any additional thoughts or to ask questions; however, these did not contribute to the data analysis regarding student definitions and notions of utility.

Data Analysis

After the data was collected from the interviews, responses to the key questions were sorted into a coding scheme that included definition and utility sections. One key question with follow-up answers was used to code for student-generated definitions. Each student response to this question was analyzed for evidence of student ideas related to the NCTM standards. These standards were split into Content and Process. Content standards include *Number & Operations*, *Algebra*, *Geometry*, *Measurement*, and *Data Analysis & Probability* (NCTM, 2023). Process standards include *Problem Solving*, *Reasoning & Proof*, *Communication*, *Connections*, and *Representations* (NCTM, 2023). The

descriptions of these standards from NCTM were used in sorting responses. Responses were then assigned a raw score out of the 10 total standards that represented the scope of the student definition. This raw scope only measured the breadth of student responses and did not represent the quality of their connections to these different standards.

Five key questions were used in the coding scheme to analyze students' notions of math's utility. Answers from these five questions were then coded, and responses were analyzed for evidence related to four utility domains using Dobie's previous research on utility value related to math education (2019b). The first utility domain was titled *Everyday Life (General)* in the coding scheme. This domain focused on broader claims about math being useful such as "Math is everywhere!" or "Math can be used in anything!" The second utility domain was titled *Future Job/Career*. The third utility domain was titled *Daily Activities*, and this domain was focused on specific examples of how math is useful in day-to-day life. Common examples were cooking, budgeting, and time management. The fourth utility domain was titled *Current or Future Schooling*. Some student connections to these utility domains were explicit, but other coding decisions were made by the judgment of the coder. Similar to the coding of responses to the definition question, responses were assigned a raw score out of 4 to represent the

4 utility domains, and this score only demonstrated the breadth of their sense of math's utility, not the thoroughness or quality of student responses.

After responses were coded, the data was analyzed to look for connections between students' math ideas of definitions and utility. Initially, connections were analyzed to see which areas of math defined by the NCTM standards were most frequently paired with which utility domains. Then, each students' breadth of math definition ideas and math utility ideas were compared to see if a wider breadth of math definition was related to a wider breadth of thinking about math's utility.

Findings

Using the selected questions from the interview and the coding scheme, students' responses were analyzed. First, the definition question was used to connect student ideas with the NCTM standards. Second, the utility questions were used to connect student ideas with the utility domains. Then, the data was analyzed together to look for connections in students' ideas about defining math and using math.

Student-Generated Definitions and Notions of Utility

Responses were analyzed for evidence of connections to the different NCTM standards. The standard with the most frequent student-made connections was *Number & Operations*, which was mentioned by 100% of participants. Students

frequently began their definitions by listing operations such as multiplication or division. Many others mentioned numbers and fractions. One student's initial response was: "I think I would say math is pretty much these numbers that you can add, subtract, multiply, or divide with and they come up with a number that you can like do some more with the number you got." The standard with the least frequent student-made connections was *Reasoning & Proof*, which was not mentioned in any of the interviews. Overall, content standards emerged with much greater frequency than process standards, as seen in Figure 1. It seems that the majority of students in the sample were more likely to imagine math as a topic and as content while fewer students imagined math as something that is practiced through processes.

Figure 1: *Frequency of Mathematical Ideas Emerging in Student Definitions*
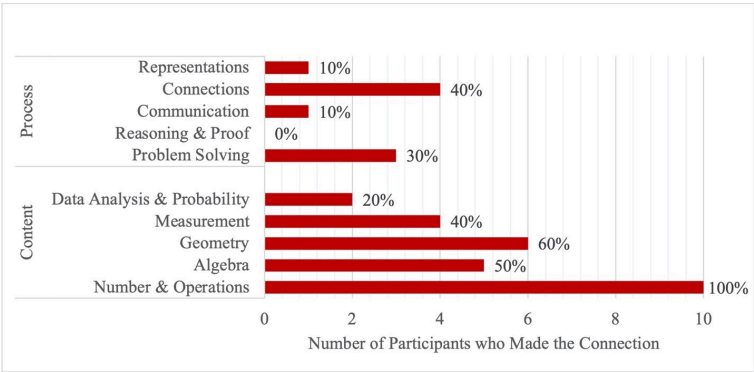
Additionally, all participating students were able to generate some ideas of math's utility, as

represented in Figure 2. The domain with the most frequent student-made connections was *Daily Activities*, which was mentioned in 100% of the interviews. Responses frequently involved money, measurements for various purposes, and time management. Related to time management, one student shared: "…when I'm trying to figure out like, uh, time management, which I struggle with a lot, so when I'm trying to plan my day, I have to do math on like, okay, estimating how much time I'm going to walk my dog, how much time I'm going to work on my homework, that kind of stuff." Some began detailing rich examples of math's usefulness in daily activities by talking about instances of specific problem solving such as with vaccines: "I believe that it is useful, and um, that you are going to need it in some scenarios because it's such a relevant thing in our society. Um, especially if you're working in jobs like engineers. And, um, people who need to know math to make our world work … people who make the vaccines, and, um, health administration. And just in general, people who, everyday people who, who come across a problem they need to solve, most of them are math-related." Connections to the utility domain *Future Job/Careers* were made in 90% of responses. The most frequently mentioned jobs and careers were cashiers, engineers, and teachers. Connections to the utility domain *Everyday Life (General)* were made in 80% of responses. These responses usually

communicated that math is simply needed to do many things like survive. One student shared: "… if they think it's not useful, like how they're gonna survive without like knowing how to multiply or like adding or something for like the real world because they're gonna need it either way." Connections to the utility domain *Current or Future Schooling* were mentioned in 70% of responses. These responses typically discussed using math in school, doing homework, or seeing other students use math for school. Many students articulated that math can be useful without any awareness that it is being used. Thus, students' sense of usefulness is not limited to their definitions of math.

Figure 2: *Frequency of Utility Domains Across Student Responses*
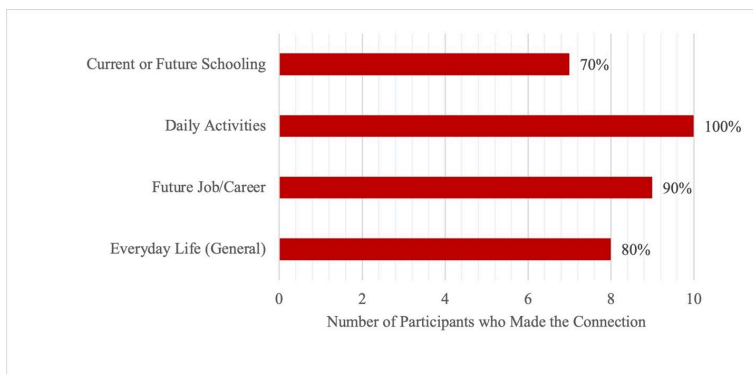
Definition and Utility Connections

After analyzing student-generated definitions and notions of utility, the ideas were looked at concurrently. This was done in two ways. First, the NCTM standards that were mentioned by each

student in response to the definition question were compared with their answers to utility questions. Some ideas related to NCTM standards were mentioned with no utility connection, but this analysis was specifically looking at the frequency of a definition area being connected to a utility domain. This analysis explores how students think of different areas of math as useful in specific ways. Second, the overall breadth in emerging ideas from students related to their definitions of math and notions of utility were compared. Their individual responses were scored comparing the breadth of NCTM standard connections and utility domains mentioned in their interview. This analysis helps us to see whether having a broader definition of math is connected with a broader sense of its utility.

The frequency of definition areas being connected to utility domains represents how different NCTM standards are perceived as useful and in what ways a certain area of math is considered useful by students. These findings can be seen in Table 1. Among the content standards, *Number & Operations* had the most connections to utility domains with 12 total mentions. Of those 12 connections to utility areas, seven were to *Daily Activities*. Not all students mentioned examples of *Number & Operations* in their interviews, but other students connected this standard to multiple utility domains. A handful of students specifically connected *Number & Operations* to both *Future Job/*

Career and *Daily Activities.* This meant that the overall utility connections made for this standard exceeded the total number of participants. *Number & Operations* having the most utility connections makes sense as it was also the most frequently mentioned NCTM standard from the definition questions. However, the definition area with the second most utility connections was only mentioned by 40% of participants, and this definition area was the NCTM standard, *Measurement.* Responses contained seven connections between *Measurement* and utility domains with four of those connections being related to *Daily Activities.* Some definition areas and NCTM standards had no utility domain connections, such as *Communication* and *Connections.*

Table 1: *NCTM Definition Areas and Most Frequent Utility Domain Connections*

Definition Category (Process)	Problem Solving	Reasoning & Proof	Communication	Connections	Representations
Number of Times Paired with Utility Connection	5	0	0	5	1
Most Common Utility Connection	Everyday Life, Future Job/Career (2 each)	N/A	N/A	Future Job/Career (2)	Future Job/Career (1)

Definition Category (Content)	Numbers & Operations	Algebra	Geometry	Measurement	Data Analysis & Probability
Number of Times Paired with Utility Connection	12	1	2	7	3
Most Common Utility Connection	Daily Activities (7)	Current or Future Schooling (1)	Current or Future Schooling, Daily Activities (1 each)	Daily Activities (4)	Future Job/Career (2)

After looking at the kinds of connections made between definition areas and utility domains, the overall breadth of definitions and notions of utility were compared for each student. Each student response was given a score for their scope of definition and a score for their scope of utility. Their responses to the definition question were scored out of 10 with a percentage.

This score reflected how many of the 10 NCTM standards emerged in their response. Students' scopes and breadth in response to the definition question ranged from 10% to 60% with a mean of 37%. Furthermore, their responses to the utility questions were scored together out of 4 with a percentage. This score represented how many connections were made to the 4 utility domains.

Students' scopes and breadth in response to the utility questions ranged from 75% to 100% with a mean of 87.5%. There were no overarching patterns in students' scope of mathematics in relation to their scope of utility notions as seen in Table 2. What was interesting from this analysis was the comparison between students with the greatest and smallest scope in definition. Both the student with the widest definition scope score (60%) and the student with the narrowest definition scope score (10%) had a 75% scope score in response to the utility questions. Thus, students' ability to explain examples of math utility was not related to their scope in definition.

Table 2: *Individual Students' Definition Breadth Score and Utility Notion Breadth Score*

	Student A	Student B	Student C	Student D	Student E	Student F	Student G	Student H	Student I	Student J
Definition Percent Score	60%	30%	40%	50%	30%	50%	30%	20%	50%	10%
Utility Percent Score	75%	75%	75%	100%	100%	100%	100%	75%	100%	75%

Discussion

These findings connect to prior research in important ways. However, we must also consider the limitations of this work. Finally, we can consider the implications of the present study for future research and for future practice.

Relation to Metzger's Work

The present study's methods of analyzing student-generated definitions of mathematics were influenced by Metzger's work (2019) to understand students' conceptions of mathematics. However, there are some important differences between these studies. First, the framing of the questions asked in the interviews to collect data on student ideas was different. In Metzger's interviews, they asked students: "What is math?" whereas in the current study, students were asked: "Imagine that a new kid moved to your neighborhood and that kid has never taken a math class before or even heard the word 'math'. Can you pretend I'm that new kid and tell me, what is math?" Additionally, the age of participants across these studies differed. Metzger's work focused on elementary aged students entering grades one through four (2019). In the

current study, middle students were the focus with participants all being in eighth grade. The sample size of these studies also differed. In the 2019 study, there were 99 participants compared to the 10 participants in the current study, and the 2019 sample had greater racial and ethnic diversity than the current study (Metzger et al., 2019). While there are some differences between the studies, important insights can still be made by comparing the two.

Results from Metzger's study showed a large majority of students focused their ideas about math on content areas of math (91.9% of responses) over processes with the majority of those responses focused on *Number & Operations* (89.9% of responses) (Metzger et al., 2019). These findings align with the current study where 100% of participants mentioned ideas related to *Number & Operations*. However, the present study had more diverse ideas emerge related to the NCTM standards than Metzger's work. Focusing on just content standards, *Algebra* was discussed by 3% of students in Metzger's study compared to 50% in the present study. *Geometry* was discussed by 8.1% of students in Metzger's study compared to 60% in the present study. *Measurement* was discussed by 7.1% of students in Metzger's study compared to 40% of students in the present study. Metzger's work did not report data on how many students mentioned *Data Analysis & Probability*, but the present study

had 20% of students mention ideas related to this standard. When looking at process standards, the present study also shows a greater percentage of students mentioning ideas related to NCTM process standards. In Metzger's work, they only reported data for *Problem Solving* and *Connections*. In their study, 5.1% of students mentioned ideas related to *Problem Solving* compared to 30% in the present study. *Connections* were discussed by 5.1% of students in Metzger's study compared to 40% of students in the present study. We can see two potential reasons for a greater scope in defining mathematics amongst the participants in the present study. First, this difference may be because students in the present study are older and have had more math education. Thus, students' conceptions of mathematics may continue to expand as they enter junior high, and these conceptions are not fixed. Second, this difference may be a result of the difference in framing the question. There is potential that the framing of the question in the present study with follow-up questions encouraged students to think about the prompt more deeply and to extend the scope of their thinking.

Limitations and Future Directions for Research

While there are practical applications for teachers and future research from this study, there are also limitations to consider. First, there are

some limitations from the study's sample. The sample size was very limited with only 10 participants. This makes it difficult to generalize the results to other contexts. Additionally, there is a lack of gender diversity in the demographics of participants.

Second, there were some limitations related to the interview process. The framing of the definition question was intentional to offer students more context and access to sharing their ideas about math. However, some of the interviews revealed potential issues with this framing. The question asks students to explain what math is to someone who had never heard of math or taken a math class. In response, one student had started to include concepts like numbers, adding, symbols, equations, and more. They were explaining these concepts in a sequence in which they thought math should be taught. They began to explain the reasoning behind this thinking: "I probably wouldn't want to instantly start teaching the dude [the imagined new student] square roots and cubic roots and division." This response spurs questions about other students who potentially interpreted the question as a way to teach math rather than define math. Perhaps the framing of the question removed students' intention to define mathematics and limited their answers to their perceptions of the more accessible parts of math. Within this study, this limitation is partially remedied by the follow-up question where

students were asked about other words and pictures that come to mind when thinking about math. However, this question does not directly prompt students with the intention to define math; it asks students to think about ideas related to math. Thus, future research that aims to understand how students define math should offer more access and launching points, but should also ask follow-up questions that allow students to attempt to define math. Future interviews may start with questions like this interview that offer launching points for students without influencing their responses with other stimuli and follow these questions with explicit questions about defining math.

Additionally, connections might have emerged in students' responses on some questions other than those selected for the present study. This means that students' scope of math's usefulness may not be totally represented in this data analysis. On the opposite end, follow-up questions that connected to other portions of the interview may have caused students to give more detail that was then coded for the data analysis. These follow-up questions might not have been offered equitably across participants. This limitation is because the data analysis was organized after the interviews were conducted. Future research that asks about the relationship between student-generated ideas, whether that be definitions and utility or other concepts, should ask these questions in a sequential order at the start of the interview without other questions in between that may influence student responses.

Third, there are some potential issues related to data analysis. One of these issues was that there was only one coder. Thus, there is no inter-rater reliability for these findings. Another issue with data analysis concerns a question selected for coding responses. This question asked about students' future career aspirations with follow-up questions about how math may be related to those careers. This greatly increased the probability that students would offer ideas in their interview that demonstrated connection to the utility domain, *Future Job/Career*. While this question and other questions did not guarantee that students would make mentions of ideas related to *Future Job/ Career*, there is a strong likelihood that student responses were influenced by the direct questioning related to this utility domain. Thus, future analyses should explore how frequently job and career connections emerged solely on questions where students were not explicitly asked about their future career interests.

The last potential issue related to data analysis was the way the breadth of students' definitions was evaluated. The way students' answers were compared to NCTM standards only accounted for whether a connection was made based on the coder's interpretation. If a student made several connections to one NCTM standard, this still only expanded their breadth score by 10% and would reflect the same breadth in definition as a student

who only offered one example. Additionally, this method did not allow any analysis of the quality of students' connections to reflect how deeply they understand how math is used in various ways. Future research should consider ways to evaluate multiple aspects of a student's definition about math including the breadth of math topics, the quantity of examples, and the quality of their thinking.

Implications for Future Research

The present study cannot make a definitive conclusion about the nature of students' conceptions about mathematics and whether they are fixed or dynamic. However, the comparison between Metzger's study and the current study suggests students are able to expand their definitions to include a broader scope of ideas in both content areas and processes of math (Metzger et al., 2019). Thus, future research should continue to inquire about student-generated definitions of math to examine how those definitions change over time, what factors change those definitions, and at what point in students' educational career their ideas are most fluid and adaptive.

Furthermore, the definition-focused questions about math in the interviews in the present study diverged from previous research. Where existing studies have asked more direct questions such as, "What is math?" or "What is maths all about?", the current study offered more context to the question

in efforts to offer a point of entry to the question to enrich students' opportunities to offer rich answers (McDonough & Sullivan, 2014; Metzger et al., 2019). This difference in setting up the interview question may have strengths and weaknesses. Thus, future research should focus on the most effective ways to collect students' conceptions about math and what they include in their personal definitions.

Implications for Practice

Teachers should informally collect information about their students' math definitions and notions of math utility. Teachers do not need to sit down and record student answers, but as they get to know their students, they can use these findings to guide in-class discussions related to math. As they move between math concepts and topics in their curriculum, they should invite students to share where they imagine they can use what they learn. This information sharing will both lead to more meaningful learning for students as they practice generating their own notions of math utility (Hulleman & Harackiewicz, 2009) and will also inform teachers about the specific utility values of the unique students in their classroom. This will allow the teacher to make instructional decisions that are most relevant to their classrooms and most individualized to their students. This is a particularly valuable change to instruction for teachers to make as it may include expanded notions of utility that have previously not been

the focus of utility interventions in math (Dobie, 2019b).

Teachers should consider how much emphasis they place on various math standards in their classrooms that may be leading to such a preference for students to discuss topics like *Number & Operations* over *Reasoning & Proof* or *Representations*. Additionally, they should think about the framing of their math instruction that leads students to think of math as a topic that is studied through content areas rather than by participating in processes, and this commonality in student thinking is supported both by the present study and by previous findings (Metzger et al., 2019). Teachers should rethink the opportunities they facilitate in their classrooms to allow students to use math in practice instead of what previous research has shown where students think about math as something done alone, at a desk, and in a math book (Johnasson & Sumpter, 2020). Metzger et al. (2019) suggest that there is a lack of meaningful math connections explicitly made for students; however, other researchers argue that students benefit more from opportunities to create their own notions of utility (Hulleman & Harackiewicz, 2009). The present study showed that students are able to generate a broad range of examples where math is useful. To build students' conceptions about math including both their definitions and sense of usefulness, instead of

making explicit math connections to real-world contexts, teachers should create opportunities for students to generate their own sense of utility related to each math topic and process they use in their classroom throughout the year. Elementary and middle-school students might need more support in thinking about possibilities since their world experiences are more limited than the participants in some of the previous studies. These opportunities may benefit students' learning in broadening their conceptions of math while simultaneously building their motivation through math's utility value (Eccles & Wigfield, 2002). Teachers should use their own expertise about their classrooms and the age groups they work with to determine what this looks like, whether it be math journaling, writing story problems, more in-class discussions, or other.

Conclusion

This research echoes previous literature and urges teachers and researchers to consider student-generated ideas. Returning to the research questions, middle school students define math primarily through its content over its processes. Math content was most frequently seen as useful through *Daily Activities*, while math processes were most frequently seen as useful in *Future Job/ Careers*. However, there was no evidence of a correlative relationship between students' math definitions and their notions of math's utility. Thus,

teachers should take every opportunity to expand their students' definitions of math while simultaneously providing opportunities for students to make personally meaningful connections with their math learning.

References

Brown, E. R., Smith, J. L., Thoman, D. B., Allen, J. M., & Muragishi, G. (2015). From bench to bedside: A communal utility value intervention to enhance students' biomedical science motivation. *Journal of Educational Psychology*, *107*(4), 1116-1135. Retrieved from http://dx.doi.org/10.1037/edu0000033

Canning, E. A., & Harackiewicz, J. M. (2015). Teach it, don't preach it: The differential effects of directly-communicated and self-generated utility–value information. *Motivation Science*, *1*(1), 47–71. Retrieved from https://doi.org/10.1037/mot0000015

Caspi, A., Gorksy, P., Nitzani-Hendel, R., Zacharia, Z., Rosenfeld, S., Berman, S., & Shildhouse,

B. (2019). Ninth-grade students' perceptions of the factors that led them to major in high school science, technology, engineering, and mathematics disciplines. *Science Education*, *103*(5), 1176-1205. Retrieved from https://doi.org/10.1002/sce.21524

Eccles, J. (2009). Who am I and what am I going to do with my life? Personal and collective identities as motivators of action. *Educational Psychologist*,

44(2), 78–89. Retrieved from https://doi.org/ 10.1080/00461520902832368

Eccles, J. S., & Wigfield, A. (2002). Motivational Beliefs, Values, and Goals. *Annual Review of Psychology*, *53*(1), 109-132. Retrieved from http://doi.org/10.1146/ annurev.psych.53.100901.135153

Dobie, T. (2019a). A sociocultural examination of utility value in mathematics: The role of interdependence in middle school students' perceptions of usefulness. In M. Hannula, G. Leder, F. Morselli, M. Vollstedt & Q. Zhang (Eds.), *Affect and Mathematics Education*, 67-88. Springer. Retrieved from https://link.springer.com/chapter/ 10.1007/978-3-030-13761-8_4

Dobie, T. (2019b). Expanding conceptions of utility: middle school students' perspectives on the usefulness of mathematics. *Mathematical Thinking and Learning*, *21*(1), 28-53. Retrieved from https://doi.org/10.1080/10986065.2019.1564969

Dweck, C. S., & Master, A. (2009). Self-theories and motivation: Students' beliefs about intelligence. In K. R. Wenzel & A. Wigfield (Eds.), *Handbook of motivation at school*, 123–140. Routledge/Taylor & Francis Group. Retrieved from https://psycnet.apa.org/record/2009-24219-007

Grossmann, I., & Varnum, M. E. W. (2011). Social Class, Culture, and Cognition. *Social Psychological and Personality Science*, *2*(1), 81–89. Retrieved from https://doi.org/10.1177/1948550610377119

Hulleman, C. S., & Harackiewicz J. M. (2009). Promoting interest and performance in high school science classes. *Science*, *326*(5958). Retrieved from https://www.science.org/doi/10.1126/science.1177067

Johansson, A. D., & Sumpter, L. (2020). Children's conceptions about mathematics and mathematics education. *Current State of Research on Mathematical Beliefs XVI.* Retrieved from https://www.researchgate.net/publication/339413153_CHILDREN%27S_CONCEPTIONS_AB OUT_MATHEMATICS_AND_MATHEMATICS_E DUCATION

Moll, L. C., Amanti, C., Neff, D., & Gonzalez, N. (1992). Funds of knowledge for teaching: using a qualitative approach to connect homes and classrooms. *Theory Into Practice*, *31*(2), 132–141. Retrieved from http://www.jstor.org/stable/1476399

McDonough, A., & Sullivan, P. (2014). *S*eeking insights into young children's beliefs about mathematics and learning. *Educational Studies in Mathematics,* *87*(3), 279–296. Retrieved from https://doi.org/10.1007/s10649-014-9565-z

Metzger, S. R.; Sonnenschien, S.; & Galindo C. (2019). Elementary-age children's conceptions about mathematics utility and their home-based mathematics engagement. *The Journal of Educational Research*, *112*(4), 431-446. Retrieved from https://doi.org/10.1080/00220671.2018.1547961

National Council of Teachers of Mathematics

(NCTM). (2000). Principles, standards, and expectations. Reston, VA: NCTM. Retrieved from https://www.nctm.org/Standards-and-   Positions/Principles-and-Standards/Principles,-Standards,-and-Expectations/

Stephens, N. M.; Markus, H. R.; & Townsend, S. S. M. (2007). Choice as an act of meaning: The case of social class. *Journal of Personality and Social Psychology, 93*(5), 814–830. Retrieved from https://doi.org/10.1037/0022-3514.93.5.814

Priniski, S. J., & Thoman, D. B. (2020). Fostering an Inclusively Relevant Mathematics Environment: The Case for Combining Social-Justice and Utility-Value Approaches. Retrieved from https://doi.org/10.31219/osf.io/wsx9d

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SECTION IV

College of Engineering

15. Propulsion of Flexible Oar Swimmers at Low Reynolds Number

Ruba Alraqibah

Faculty Mentor: Yong Lin Kong (Mechanical Engineering, University of Utah)

When I joined Dr. Kong's lab as a biomedical engineering major in the Spring 2021 semester, I was feeling apprehensive about my ability to contribute to a mechanical engineering lab. But to my pleasant surprise, Dr. Kong was exceptionally welcoming and encouraging. Since then, my experience at the additive manufacturing lab has been incredibly rewarding and provided me with the tools needed to succeed in research.

While in the lab, I attended group meetings where I got the chance to present and receive feedback from Dr. Kong and graduate students. I also attended meetings with our collaborators at Santa Clara University to present our findings

and discuss the progress of the project. Dr. Kong has been an outstanding mentor to me as he is always willing to answer all my questions and provide me with the feedback and guidance I need.

I have been fortunate to participate in the Undergraduate Research Opportunity Program (UROP) which acknowledged my participation in research and provided funding for my research time. Participating in UROP has also provided me with the chance to present my research at the Utah Conference of Undergraduate Research which is an exciting opportunity to increase visibility towards the impact of research.

Dr. Kong has also been my supervisor for my biomedical engineering senior thesis project. The invaluable support I received from him, and the lab members have encouraged me to grow as the student researcher I aim to be. I was able to work independently on experiments which provided me with many crucial skills in research.

When I first started working at the lab, I was trained to use SolidWorks, a 3D computer design software. I also learned about different aspects of 3D printing, other fab approaches, and troubleshooting techniques. The hands-on guidance I received was instrumental to my engineering career, as I was able to apply what I learned in the lab to my BioDesign senior capstone project as well as my industry internships.

Overall, my experience in the additive manufacturing lab has been invaluable. It has allowed me to gain a deeper understanding of how to navigate challenges that arose during my research. My experience conducting research in the past two years has provided me with a deeper understanding of designing and carrying out experiments. When things do not go to plan, my lab mentors encouraged me to identify problems that arise during an experiment, such as signal processing

errors and noise, and come up with effective solutions. I believe that the knowledge and skills learned during my time here will be both critical and beneficial to me as I move forward in my engineering career. I am grateful to be a part of this opportunity and to have had access to incredible mentors that played a crucial role in shaping my research experience.

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16. **Research Reflection by Ruba Alraqibah**

Ruba Alraqibah

Faculty Mentor: Yon Lin Kong (Mechanical Engineering, University of Utah)

When I joined Dr. Kong's lab as a biomedical engineering major in the Spring 2021 semester, I was feeling apprehensive about my ability to contribute to a mechanical engineering lab. But to my pleasant surprise, Dr. Kong was exceptionally welcoming and encouraging. Since then, my experience at the additive manufacturing lab has been incredibly rewarding and provided me with the tools needed to succeed in research. While in the lab, I attended group meetings where I got the chance to present and receive feedback from Dr. Kong and graduate students. I also attended meetings with our collaborators at Santa Clara University to present our findings and discuss the progress of the project. Dr. Kong has been an outstanding mentor to me as he is always willing to answer all my questions and provide me with the feedback and guidance

I need. I have been fortunate to participate in the Undergraduate Research Opportunity Program (UROP) which acknowledged my participation in research and provided funding for my research time. Participating in UROP has also provided me with the chance to present my research at the Utah Conference of Undergraduate Research which is an exciting opportunity to increase visibility towards the impact of research. Dr. Kong has also been my supervisor for my biomedical engineering senior thesis project. The invaluable support I received from him, and the lab members have encouraged me to grow as the student researcher I aim to be. I was able to work independently on experiments which provided me with many crucial skills in research. When I first started working at the lab, I was trained to use SolidWorks, a 3D computer-aided design software. I also learned about different aspects of 3D printing, other fabrication approaches, and troubleshooting techniques. The hands-on guidance I received was instrumental to my engineering career, as I was able to apply what I learned in the lab to my BioDesign senior capstone project as well as my industry internships. Overall, my experience in the additive manufacturing lab has been invaluable. It has allowed me to gain a deeper understanding of how to navigate challenges that arose during my research. My experience conducting research in the past two years has provided me with a deeper understanding of designing and carrying out experiments. When things do not go to plan, my lab mentors encouraged me to identify problems that arise during an experiment, such as signal processing errors and noise, and come up with effective solutions. I believe that the knowledge and skills learned during my time here will be both critical and beneficial to me as I move forward in my engineering career. I am grateful to be a part of this

opportunity and to have had access to incredible mentors that played a crucial role in shaping my research experience.

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17. Development of a Low-Cost, 3D Printed, Aspirated Air Temperature Measurement Radiation Shield

Alexis DeFord; Eric Pardyjak (Mechanical Engineering); and Rob Stoll (Mechanical Engineering)

Faculty Mentors: Eric Pardyjak and Rob Stoll (Mechanical Engineering, University of Utah)

Accurate measurement of ambient air temperature is critical to numerous applications. This task is complicated by solar radiation which can heat the air-temperature sensor body, causing it to record temperatures in excess of the true air

temperature. The traditional way to protect against this interference is to house the sensors in passive radiation shields which block the majority of solar radiation. However, past research indicates that significant solar radiation can still penetrate shield designs, causing measurement errors under low-wind conditions. An alternative method is to aspirate the sensor using a fan to force air movement over the sensor body. Aspirated temperature sensing units are commercially available, but they can be expensive and consume a significant amount of power. We designed and rigorously tested a low-cost, low-power aspirated temperature sensing unit designed to integrate with any low-cost distributed sensor platform. The design uses a freely available, custom designed 3D printed housing that enables rapid assembly. The new, open source unit performed as well as passive shields and commercial aspirated shields of significantly higher cost. This success shows the potential of leveraging 3D printing technologies for designing other housing units.
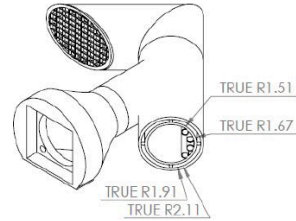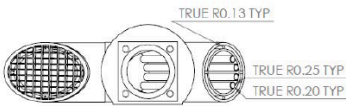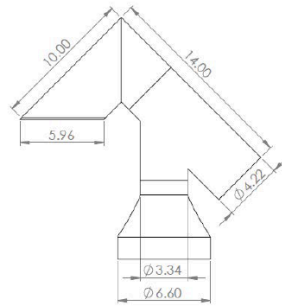
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18. **Research Reflection by Alexis DeFord**

Alexis DeFord

Faculty Mentors: Eric Pardyjak and Rob Stoll (Mechanical Engineering, University of Utah)

I joined Dr. Amanda Smith's lab as a freshman on campus, so I joined research very early on. The following year I moved to the Environmental Fluid Dynamics lab under Dr. Eric Pardyjak and Dr. Rob Stoll, and I worked with them for the rest of my undergraduate career. The experiences I've had with these labs have been wonderful; I've had fun and learned a lot, but moreover I've grown as a person. Being in these labs I've had mentors both on campus and beyond through the lab directors' connections. All of my internships have come through the networks I made in these labs. Having an outlet to use the classroom skills in a more sandbox way has certainly enriched my learning as an undergraduate. Being in research has really made me consider graduate school and perhaps even an

academic career. I welcome and am pursuing further research work primarily because of my undergraduate research experience.

About the Author

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19. Colorimetric Assay for Pneumonia Screening Via the Volatile Organic Breath Biomarker, Heptane

Bailey Doucette

Faculty Mentor: Swomitra Mohanty (Chemical Engineering, University of Utah)

Pneumonia is the single largest infectious cause of death in children worldwide. Diagnosing pneumonia through a rapid and proactive method would increase the speed at which patients are treated and can recover. Volatile organic biomarkers (VOBs) are present in the breath of patients

infected with pneumonia. One such VOB is heptane, which has been proven to be present in the breath of pneumonia patients. Pneumonia is usually diagnosed via chest X-ray, an expensive method of examination. This method of diagnosis is done after patients begin to exhibit symptoms. By engineering an inexpensive device that would detect heptane from the breath in an efficient way through colorimetry, patients could be diagnosed before symptom onset and treatments could start before damage could be done. To confirm the presence of heptane as one of the VOBs associated with pneumonia, staphylococcus aureus was cultured in our laboratory and the released VOBs were measured using GCMS. Heptane is of particular interest as it reacts with water bromine when exposed to UV light in a halogenation reaction. The water bromine loses its characteristic orange-red color during the reaction. Thus, this reaction can detect heptane in patients' breaths in a point-of-care Pneumonia screening device. Colorimetric methods are used to analyze the concentrations of compounds. The concentration of a colored compound can be determined with a spectrophotometer. Utilizing a blue LED and an OPT101 monolithic photodiode, the concentration over time of water bromine within the water bromine heptane reaction was attained in a spectrophotometer. The engineered spectrophotometer in the initial part of this project was designed to run the heptane water bromine reaction within a cuvette between a blue LED and OPT101 monolithic photodiode. The spectrophotometer was characterized via calibration and used to detect concentration changes from the water bromine and heptane reaction.

About the Author

Bailey Doucette
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20. Effectiveness of Ski Wax Treatments in Sintered Ski Bases Using Different Temperatures and Application Methods of Ski Wax

Lindi A. Hopkins

Faculty Mentor: Jeffery S. Bates (Materials Science and Engineering, University of Utah)

This project has focused on application temperatures and methods on sintered ski bases. It is believed that sintered bases have a higher porosity, allowing for better absorption of ski wax. There is a tradeoff with this method as it is more expensive to produce than extruded bases, sintered bases are generally reserved for higher performance skis, such as those used by alpine racers and heavily experienced hobbyists. Ski waxes have been formulated for different conditions of snow primarily cold, warm, and all-temperature waxes. While there has been extensive testing of these waxes' characteristics, there has not been extensive research on varying application methods and subsequent temperatures. This project delved into application methods and characterization of ski base samples using tribology, hardness, and contact angle testing to obtain data that can be translated into a more effective wax for consumers. Tribology measures the coefficient of friction between the base of the ski and the snow. Results from tribology testing can determine mechanical and thermal stability of the base, this testing could be enhanced by the use of a rheometer that tests the coefficient of friction as a function of temperature. Hardness testing, specifically Shore A hardness, tests hardness as a function of temperature which can give insight into how ski wax hardens onto the base. Contact angle can be obtained using the Sessile drop technique, this can help describe the friction factor snow has on sliding velocity, surface roughness, and surface pattern. There are many types of ski wax, recently a large number of brands have stopped producing fluorinated wax as there has been recent environmental concerns revolving around PFOA found in fluorinated waxes. This project utilizes waxes without PFOA to stay relevant with the current transition. In addition, there are many waxing techniques, which can vary from application

temperature to the process in base treatment before and after waxing.

About the Author

Lindi Hopkins
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21. Research Reflection by Lindi Hopkins

Lindi A. Hopkins

Faculty Mentor: Jeffrey Bates (Mechanical Engineering, University of Utah)

My experience in undergraduate research was wonderful! It allowed me to explore the world of research while working closely with a mentor who understood my goals and what I wanted to accomplish. I will carry the things I learned into my future jobs and fondly remember my time spent working in the lab.

About the Author

Lindi Hopkins
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22. Investigation of Contaminants In Stormwater Entering Red Butte Creek from University of Utah

Wona Kim and Jennifer Weidhaas (Civil and Environmental Engineering)

If people gather to create an environment for humans in a place where there are only trees, rivers, and animals, the population density will increase, thus increasing the possibility of contamination of nearby rivers and streams [1]. The location

of my research, Red Butte Creek, is located in Salt Lake City, Utah. Red Butte Creek is a river flowing in a natural area with few sources of pollution. The source of pollution can be the rainwater that passed from the University of Utah campus. This water runoff can lead to contamination of the Jordan River and the Great Salt Lake [2]. However, as time passed and human influences began to pollute the water quality. It is necessary to manage and preserve this place through continuous measurement and research of water pollution [3]. Weather data in Salt Lake City shows that from October to March, snowfall ranges from 0.2 inches to 0.6 inches [4]. Water that melts from snow or rain is called stormwater, and it causes problems whenever the stormwater carries pollutants from human activities. For example, the pollutants may happen from parking lots, buildings, fertilizers on lawns, and excess sediment or leaves. The pollutants from these stormwaters are not only harmful to the environment but also to the humans living around them. The project I worked on is a water quality test and results at Red Butte Creek into which polluted stormwater ran.

Figure 1: Location of Red Butte Creek Storm Drain
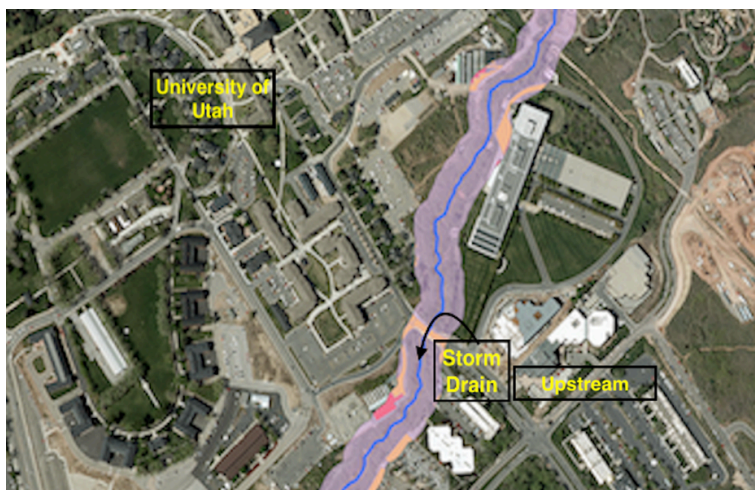
METHODS

Sampling Sites

Two water sample collection sites are shown in figure 1 which are Storm Drain and Upstream. Samples were collected above the stormwater outfall, from within the stormwater outfall, and below the stormwater outfall where the stream water is well mixed with the stormwater. They were collected twice a week for 5 weeks, a total of 9 samples. The sampling sites were in different conditions according to the weather since the research progressed during Summer. If the temperature at the creek was higher than 12°C, it had difficulty getting stormwater because of its dryness. Also, sample collection was varied based on rainfall during this period.

Sample Collection Methods

Water samples were collected at the site mentioned above with autoclaved bottles. Bottles were rinsed 2 times with each site's water to remove factors that may change the experimental results. Then 2 bottles were filled with 1L of each sample by hand. Temperature, pH, and electrical conductivity were recorded from the site. Samples were collected on May

25th 2022, June 1st, June 8th, June 15th, June 22th, June 29th, July 6th, July 13th, and July 20th. Samples were not collected during periods of the rainy season and heat waves.

Analytical Methods

The methods used for this research were pH, E.coli, Turbidity, and TDS(Total Dissolved Solid). Turbidity and pH were measured again in the lab by each meter as first soon as possible to prevent any changes as time passes. The TDS was measured by gravimetric methods after filtration onto a 0.45 um filter. E. coli was measured by culture-based methods using EPA method 1603. For E.coli experiments, samples were diluted at a 1:1 ratio. Then, isolate heatresistant E.coli from water using pre-prepared m-TEC Agar filtration technology. After the Autoclave procedure, samples are identified using urea substrate to identify bacteria.

RESULTS

Figure 1 indicates the results for each parameter which were measured in this research. As the time approached August, the temperature of the site increased and the amount of water in the creek decreased. Except for the Temperature graph, the blue color indicates the measured value of Stormwater. On July 21st, the Storm Drain was not tested since there was not enough water to run the experiment. pH values of Storm Drain are all low compared to Upstream water. About pH of 6.5-8.5 is appropriate for the creek to maintain its conditions, and most of the pH values are located between the indicated values [5]. According to the graph of turbidity in Figure 1, Upstream has relatively high values than Stormwater. The relationship between turbidity and stream flow can be one of the reasons for this result. Turbidity refers to the degree of turbidity of water containing suspended matter, which can determine the amount of sediment or nutrients and bacteria. Since both E.coli and

turbidity parameters measure the matter contained in water, these are proportional Therefore, comparing the two graphs in Figure 1, it has a similar trend line when errors that may occur during the experiment are excluded.

Figure 2. Graphs of Each Parameter
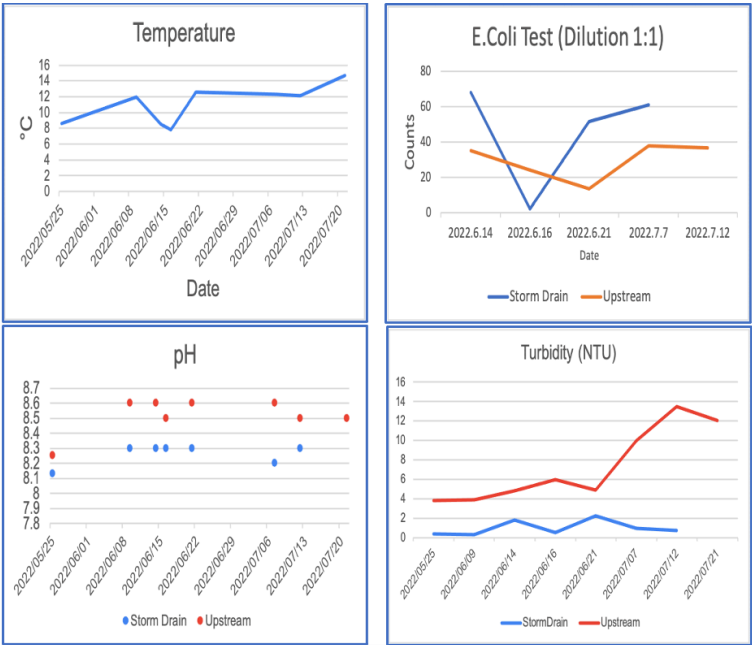
Table 1. Average & Standard Deviation for Each Parameter Parameter

Parameter		Average	Standard Deviation
Temperature		11.08	2.45
Amount of Rainfall last week		0.4	0.1
Discharge in Stream		2.09	0.77
pH	Storm	8.26	0.07
	Upstream	8.52	0.12
Electrical Conductivity	Storm	1.46	0.66
	Upstream	0.60	0.04
TDS	Storm	972.86	386.12
	Upstream	407.50	27.12
Turbidity	Storm	0.97	0.75
	Upstream	7.36	3.89

Table 2. t-Test and p Value Between the Upstream and Storm Drain Samples

Parameter	t-Test	P Value	Stasticially Significant
pH	Equal Variances	0.0002	O
Electricial Conductivity	Unequal Variances	0.0144	O
E.coli	Equal Variances	0.2891	X
TDS	Unequal Variances	0.0083	O
Turbidity	Unequal Variances	0.0019	O

Both of the tables are to compare the values between the Storm Drain and Upstream. The values for Storm Drain with pH and TDS were higher than Upstream, and Electrical Conductivity and Turbidity values were lower than Upstream. A p-value of 0.05 or lower is considered statistically significant. According to Table 2, all parameters were statistically significant except for E.coli which had a higher value than 0.05.

CONCLUSION

The average pH and turbidity values of the Storm Drain are less than the Upstream while average electrical conductivity and TDS values were higher than the Upstream. These data result indicates that the stream is more contaminated after the Storm Drain input. Because the flow rate upstream is higher than in the storm drain, the measured turbidity values are larger. Changes can occur because of various chemicals and pollutants when passing through a pipe.

REFERENCES

[1] Giddings, E. (2000). Water quality and macroinvertebrate

communities of emigration and Red Butte Creeks, Salt Lake County, Utah. Fact Sheet. https://doi.org/10.3133/fs16100

[2] Red Butte Canyon Research Natural Area. (n.d.). Retrieved March 7, 2022, from https://redbuttecanyon.net/

[3] Liyanage, C., & Yamada, K. (2017). Impact of population growth on the water quality of natural water bodies. Sustainability, 9(8), 1405. https://doi.org/10.3390/su9081405

[4] US Department of Commerce, N. O. A. A. (2022, January 24). Climate. Retrieved March 2, 2022, from https://www.weather.gov/wrh/Climate?wfo=slc

[5] Water quality. (n.d.). Retrieved February 6, 2023, from http://www.cotf.edu/ete/modules/waterq3/
WQassess3a.html#:~:text=Most%20streams%20have
%20a%20neutral,8.6%20may%20be%20too%20basic.

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23. **Disabled, Not Disqualified: Ableism in Recruitment and Retention in Game Development**

Will Loxley

Faculty Mentors: Fernando Rodríguez and Ashley Guajardo (Entertainment Arts Engineering, University of Utah)

Abstract

Game development careers are widely regarded as turbulent due to a fiercely competitive barrier of entry and industry reliance on harmful labor practices. Periods of compulsory overtime known as crunch plague professional game developers, while prospective developers seeking entry-level employment are challenged by a critical lack of transparency

from game companies. And despite disabled individuals being especially vulnerable to discrimination from recruitment and retention practices, the practical experiences of disabled game developers both in-and-outside the industry are significantly underexplored.

This paper documents how AAA game companies publicly and actively recruit disabled people as candidates for the next generation of the games industry – if at all. An institutional ethnography is conducted to investigate if game company recruitment efforts operate under the incorrect assumption that recruitment is an inherently neutral or objective practice. The research centers disabled game developers through the inductive thematic analysis of 20 AAA game companies' public facing documentation via document analysis to determine if disabled people are excluded from being talent recruitment.

Game companies are found to disregard disabled candidates in recruitment efforts and public facing documentation exhibits a low level of support for disabled professional game developers. The results suggest high-level improvements for online game company recruitment practices so as to better represent internal values and diversify the potential pool of applicants. The results also inform future research on inclusive hiring programs and identifying industry norms which negatively affect recruitment and retention for disabled game developers.

Background

Game development is a "creative collaborative practice" (O'Donnell 2014) in which technical and creative disciplines regularly interact to deliver a minimum viable product, often under extreme project management constraints. "Triple-A" (AAA) is the conventional face of game development, as AAA games are typically regarded as blockbusters due to

consistently high levels of popularity. The disciplines involved in development are progressively refined as new cohorts of game developers (GD) attempt to improve and remain active in the industry, despite the uniquely paced and passion-oriented organization of work (Cote & Harris 2021; Taylor 2006). Maintaining GD careers is thus vital to the overall health of such a high-commitment and high-involvement industry (Weststar & Legault 2017), particularly for underemployed communities.

Although several historically marginalized communities qualify as underemployed in the games industry, the overwhelming majority of research in games spaces directed toward disability pertains to disabled users, or players, in the form of frontend digital accessibility. Further, research on equitable product design and digital games labor has trended toward focusing on non-disabled working lives, whether intentionally or otherwise. This has resulted in dramatically less research examining ableism in the industry itself compared to other instances of minority discrimination. Such a consistent disregard for disability begs the question: are disabled people seen exclusively as players rather than contributors to the next generation of the games industry?

Critical disability studies coined the social model of disability, which affirms that individuals are disabled by a societal context as well as an individual's physical or mental condition (Hahn 1985). As a result, disability can be a created "social product" (Fougeyrollas et al. 2019). Disabling situations are thus the specific societal contexts in which disability is reinforced (Hamonet & Gracies 2013). If 25% of professional GDs disclose a disabled identity (IGDA 2019) and are regularly exposed to extreme and demanding working conditions, there

is then substantial risk for disabling situations to occur within game development.

The games industry is infamous for relying on periods of compulsory overtime, otherwise known as crunch (Cote & Harris 2020). GDs who crunch operate under significant duress and may experience psychosomatic health issues and degraded work satisfaction (Niemelä 2021). Disabled GDs are especially vulnerable to such effects due to disabling situations resulting from a potentially decreased capacity for working overtime (Walls & Batiste 1996) or manifestations of external and internalized ableism, such as forced disclosure of disability (Charmaz 2010). Nevertheless, the number of GDs reporting recent use of crunch has nearly doubled in just two years (IGDA 2021). Crunch has been a continuously popular topic of study in gaming circles throughout the 2000s (Dyer-Witheford & de Peuter 2006); unsurprisingly however, the disabled GD experience is explored significantly less – both in regards to crunch, and in the industry as a whole.

Game company values are reflected in the practical realities of development (Flanagan 2009), including recruitment. While interpersonal interactions in work environments can be discriminatory, disabled people also experience ableism from inaccessible systems related to human resources (Reber et al. 2022). As we continue to survive a global pandemic and mass disabling event, inaccessible recruitment practices will prevent exponentially more talent from succeeding in the games industry.

Inaccessible recruitment practices can be characterized as microaggressive (Keller & Galgay 2010) and operate under key assumptions that a candidate adheres to the following traits: a candidate is obviously able-bodied (Baert 2016; Scholz 2020), and would disclose being disabled (Ameri et al. 2017); a

candidate is or will be perceived by coworkers as productive (Østerud 2022; McLaughlin et al. 2004); and a candidate is not or will not be perceived by coworkers as a distinct or separate category of worker (Kwon & Archer 2022; Mik-Meyer 2016). It is likewise assumed by employers that they are or would be more compelled to hire a disabled candidate than other employers (Andersson et al. 2015). And even if a game company were to avoid these key assumptions, recruitment practices may still fail to account for invisible disabilities (Kattari et al. 2018; Syma 2019), such as cognitive disabilities and chronic illness.

The means by which GD careers are established and maintained are core to improving practical realities in game development. Just as consumer-focused disciplines iterate in the pursuit of a final shippable product (e.g. the design discipline of games user experience), recruitment practices should follow a similar pattern of iteration in such a dynamic labor market. Yet, the norms of secrecy and limited information flow have led to little being publicly available to prospective developers regarding the accessibility of game development (Kulik et al. 2021).

Related industry norms, including resistance to work-from-home accommodations or forced return-to-office policies, as well as the aforementioned crunch, are no longer strictly topics of research and discussion; instead, norms are being challenged by a burgeoning labor rights movement. The first successful pushes for unionization amongst GDs are emblems of unprecedented momentum (Weststar & Legault 2019). Now more than ever, the accessibility of game development is of collective industry interest (Cote & Harris 2020; Das et al. 2021) and disabled perspectives are salient.

A Disabled Perspective

As an autistic, chronically ill student GD with ADHD, I began this research out of frustration. After years of emails containing "impressed" or "unfortunately," and rejections that always came despite seemingly positive rounds of interviews, I was eager to improve as a developer. I wanted to learn everything I could from contributing to live projects, but I eventually discovered there is a limit to what we can do on our own. I became concerned: was my potential as a disabled candidate being artificially limited in a competitive and demanding job market?

An overall lack of industry support for disabled prospective GDs like myself made breaking into the games industry seem impossible. Common pieces of advice that I observed in game development spheres pertained to either: networking through massively inaccessible in-person industry meet-ups in an ongoing pandemic; or an underlying assumption that disabled candidates must have the same energy levels and time available as our non-disabled counterparts to dedicate toward job applications.

I noticed impressive and successful diversity efforts being directed to many underemployed communities, meanwhile disabled people were practically left to fend for ourselves. It became evident that diversity includes disability, but game companies might not. I spent hours meeting with every professional who was willing to offer feedback or advice. And I realized I could examine the current landscape to hopefully encourage others like me that they are not alone in their quest to enter an unaccommodating, rigorous industry.

As a first step toward emphasizing the disabled GD experience, the following pilot study was designed to illustrate the level of consideration for disabled candidates exhibited by

game companies – including those which comparatively excel in considering other marginalized identities.

Research Design

This study proposed that disabled candidates were not being sufficiently equipped to successfully enter and succeed in the games industry. It was hypothesized that game company recruitment efforts operate under an incorrect assumption where recruitment is an inherently neutral or objective practice, rather than a field subject to ableism in companies' search for an ideal candidate. It was consequentially proposed that disabled people were being excluded from talent recruitment (Hoque & Bacon 2021). It was further hypothesized that game companies specifically addressing disability, rather than general diversity, was uncommon. Disabled GDs were ethnographically centered through an inductive thematic analysis of 20 AAA game companies' public facing documentation to determine if disabled people are functionally excluded from being talent recruitment.

The game companies selected were the first AAA studios the disabled GD and researcher applied to as a student seeking entry-level employment. Data was collected in the form of compiling screenshots and excerpts from game company websites. This study qualified a game company website as a career hub or page. Some companies had singular all-purpose websites, which qualified for this study by default. The order in which data was collected was directly informed by each website's layout, as the intent was that a member of the websites' intended audience would follow the perceived user experience flow.

Consider Nintendo of America's website as an example. Nintendo's landing page features quick navigation to site content at the top, but also directs users to the following pages

by scrolling down the landing page itself. Data was thus collected from Nintendo of America in the following order: "Benefits & Perks" (Image 1.1), "Life at Nintendo" (Image 1.2), "Diversity, Equity & Inclusion" (Image 1.3), and finally "About Us" (Image 1.4).

Image 1.1 – Nintendo of America "Benefits & Perks"

BENEFITS & PERKS

Reach your goals

We invest in your health and wellness with a variety of benefits, so you can level up in all areas of your life.

See what's included

Image 1.2 – Nintendo of America "Life at Nintendo"

LIFE AT NINTENDO

Get serious about fun

Find out how we set our employees up for success —and see what it's like working with these characters.

Come on in
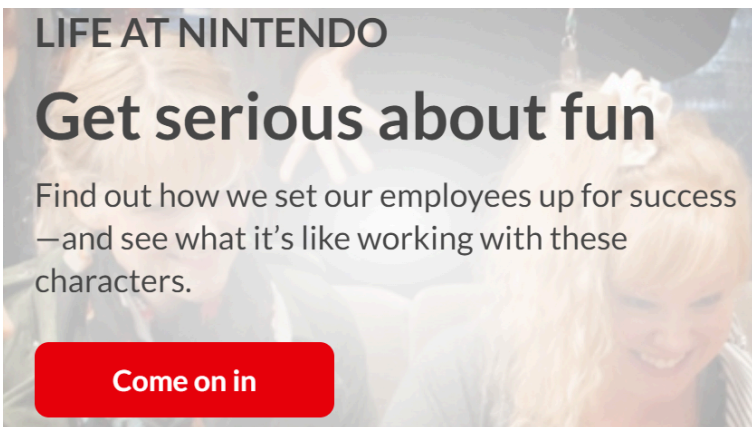
Image 1.3 – Nintendo of America "Diversity, Equity & Inclusion"

DIVERSITY, EQUITY & INCLUSION

Nintendo is for everyone

As a global company, we value diverse perspectives. Find out how we put our values to work.

Read on
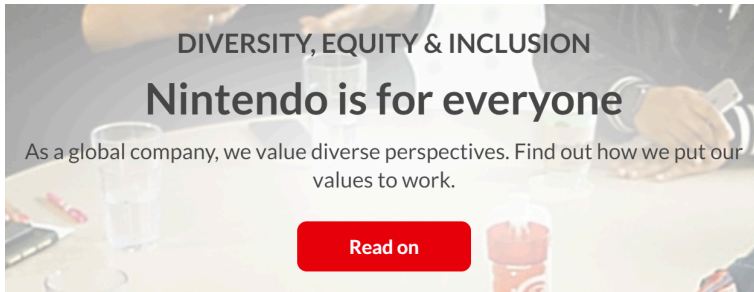
Image 1.4 – Nintendo of America "About Us"

ABOUT US

Hi, we're Nintendo.

Learn a little about where we're from and where we're going.

Go now
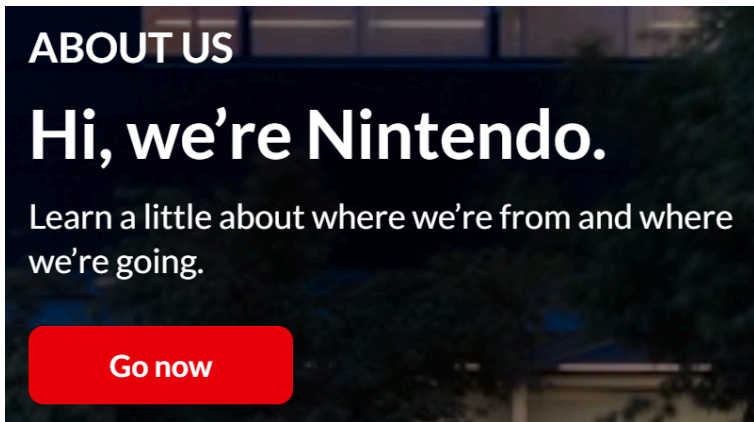
The 20 AAA game company websites were accessed for data collection from November 2022 to December 2022 in the following randomized order (Figure 1).

Figure 1. AAA Game companies examined and website URLs

Order Accessed	Game Company Name	Game Company Website URL
#1	Bungie	https://careers.bungie.com/
#2	Blizzard Entertainment	https://careers.blizzard.com/
#3	Activision Blizzard	https://careers.activisionblizzard.com/
#4	Activision	https://careers.activision.com/
#5	Wizards of the Coast	https://company.wizards.com/
#6	Riot Games	https://www.riotgames.com/
#7	PlayStation	https://www.playstation.com/en-us/corporate/playstation-careers/
#8	Electronic Arts	https://www.ea.com/
#9	Ubisoft	https://www.ubisoft.com/en-us/company/about-us/
#10	2K Games	https://2k.com/en-us/about-us/
#11	Nintendo of America	https://careers.nintendo.com/
#12	Epic Games	https://www.epicgames.com/site/en-us/about/
#13	Insomniac Games	https://insomniac.games/careers/
#14	Respawn Entertainment	https://www.respawn.com/studio/
#15	Gearbox Software	https://www.gearboxsoftware.com/about/
#16	Sony Santa Monica	https://sms.playstation.com/who-we-are/
#17	Microsoft	https://careers.microsoft.com/us/en/
#18	BioWare	https://www.bioware.com/
#19	Turn 10 Studios	https://turn10studios.com/careers/
#20	King	https://careers.king.com/

Data was organized under five classifications: Taglines, identified by mission or vision statements (Image 2.1).

Image 2.1. Blizzard Entertainment, example tagline

We're on a quest: bring our dreams to life and craft the most epic entertainment experiences, ever. Hard work, iteration and polish go into the Blizzard "secret recipe," but the most important ingredients come from talented people who share our vision. If that sounds like you, join us.

Equal opportunity disclosures, identified by legal employment notices (Image 2.2).

Image 2.2. Activision Blizzard, example equal opportunity disclosure

Core values, identified by "we" action statements, such as "we value" or "we hold" (Image 2.3).

Insert Image 2.3. King, example core values

We are seriously playful: we balance art and science, we are supportive but demanding in everything we do.

As a result, we are building a company where everyone can thrive, are proud to belong, hold each other accountable and where we manage our business for sustainable growth.

Culture statements, identified as internally focused taglines which emphasize recruitment, including information on locale, etc. (Image 2.4).

Insert Image 2.4. Electronic Arts, example culture statements

We believe in the potential of every human being

We celebrate diversity of thought, cultural differences, lifestyle, age, background, experience, religion, economic and social status, gender identity, and marital status among other aspects.

We invest in DEI across our business to empower our people, actively foster inclusion in our communities and shape the future of Electronic Arts.

And closers, identified as information placed immediately before or nearby website elements which redirect users to job listings (Image 2.5).

Insert Image 2.5. Epic Games, example closer
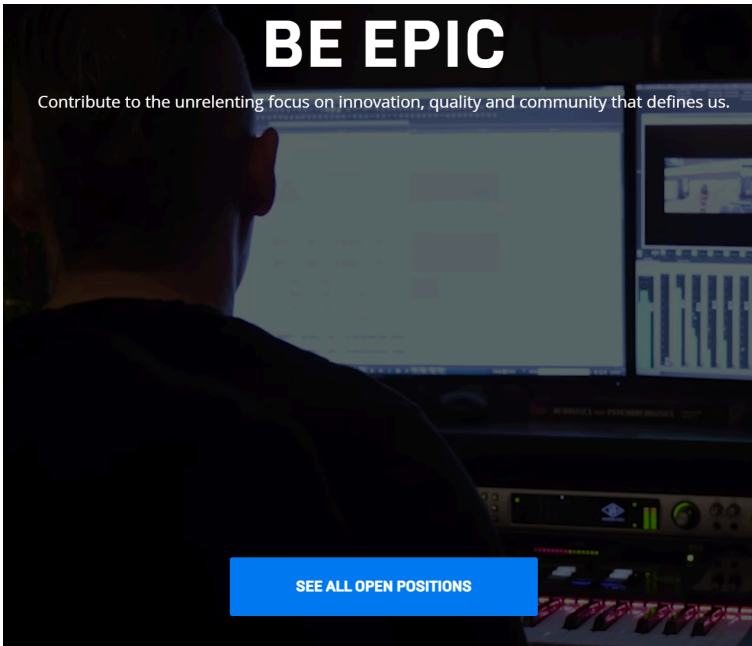
Results

Through thematic document analysis, there were four prevailing themes throughout the 20 game companies examined. Theme 1 was Recruiter Info, which gauged if the game company website directed candidates to recruiter information, such as a point of contact email, recruitment-focused Twitter, or LinkedIn (Image 3). Theme 1 was primarily present in culture statements and closers. 55% of game companies directed users to recruiter information (Figure 2).

Image 3. Activision, example of Theme 1 present

FAQs

Do you have to be a "gamer" to work at Activision?
No! To deliver epic entertainment experiences, we must bring the most visionary creative talent and the most capable business talent together. We believe that independence and the freedom to approach things differently are the keys to making groundbreaking entertainment. What makes Activision different than other gaming and entertainment companies is our belief that you've gotta have fun to make fun. When you love what you do, we believe more people will love what we make.

How does Activision give back to the community?
We provide our employees with several different ways to give back, from mentoring at-risk students and supporting the advancement of STEAM education, through programs geared towards supporting the veteran community and their transition to civilian life. Our interns have the opportunity to participate in these programs during their time at Activision, but we also offer intern-specific giving back opportunities for those who would like to join.

I still have questions; who can I ask?
You can reach out to our team directly at campus.recruiting@activision.com for more information. Please allow 48-hours for a response.

Theme 2 was DEI Values (Diversity, Equity, and Inclusion), which gauged if company culture statements specified DEI-oriented values (Image 4). DEI-oriented was defined as something directed toward conventional benefactors of diversity, equity, and inclusion practices – including historically marginalized communities (e.g. Black, Brown, Indigenous, Asian, queer, trans, gender-nonconforming, disabled, neurodiverse, etc.). Theme 2 was primarily present in taglines, equal opportunity disclosures, and core values. 60% of game companies specified DEI-oriented values (Figure 2).

Image 4. Bungie, example of Theme 2 present

Widen Your Perspective

Everyone should feel welcome in our studio and in our games. So we work hard to recognize our unconscious biases, amplify underrepresented voices, question the status quo, and to always assume the best of one another.

Theme 3 was DEI Examples, which gauged if game companies provided examples of DEI-oriented values in practice (Image 5). Examples included employee resource groups (ERG) and company DEI reports. Theme 3 was primarily present in core values and culture statements. If Theme 2 was present for a game company website, Theme 3 was also present. If Theme 2 was not present, Theme 3 and Theme 4 were also not present. The same 60% of game companies which specified DEI-oriented values also provided examples of DEI-oriented values in practice (Figure 2).

Image 5. PlayStation, example of Theme 3 present

Flex Modes

Sony Interactive Entertainment offers a global hybrid office/remote working model — Flex Modes — which aims to combine the flexibility of remote working with the magic of in-person collaboration. The model is built upon the principle of working together in-person as work requires, with the freedom to work remotely otherwise.

Our ambition is that Flex Modes addresses both the needs of the individual and the needs of the business, while strengthening our collaboration, connections and culture.

Theme 4 was Disability Specified, which gauged if disability is included in provided examples of DEI-oriented values in

practice (Image 6). Theme 4 was present in culture statements, mainly in the form of ERGs. 35% of game companies specifically referenced disability (Figure 2). Only 10% of game companies had all four themes present, and only 10% had no themes present.
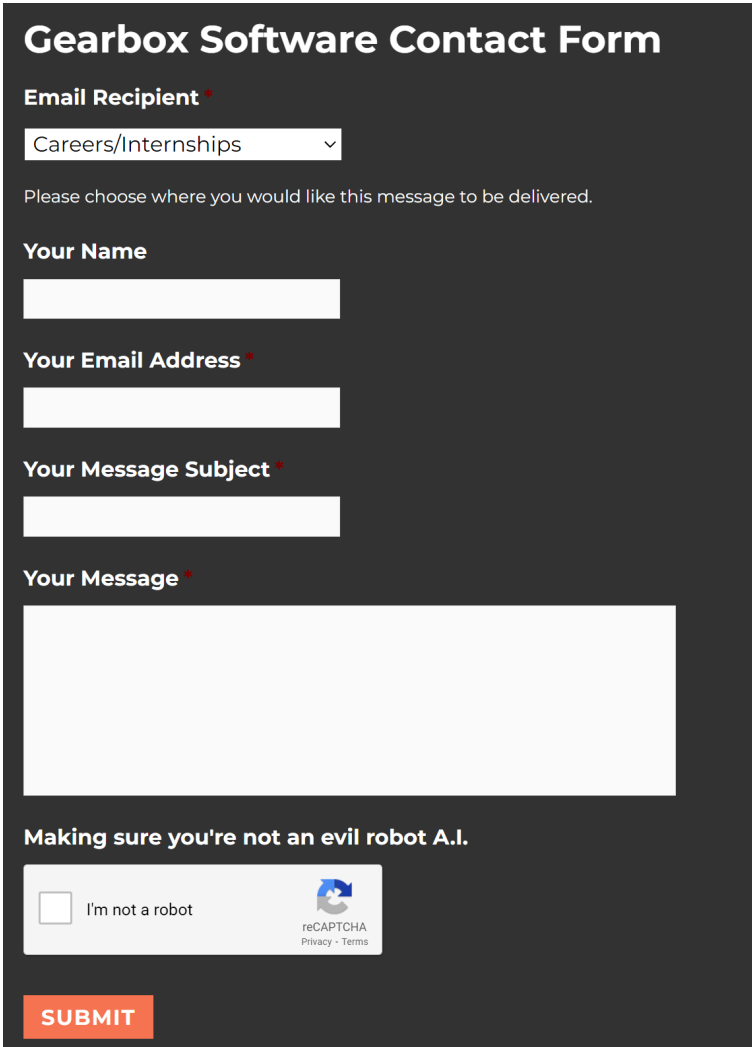
Image 6. Activision Blizzard, example of Theme 4 present
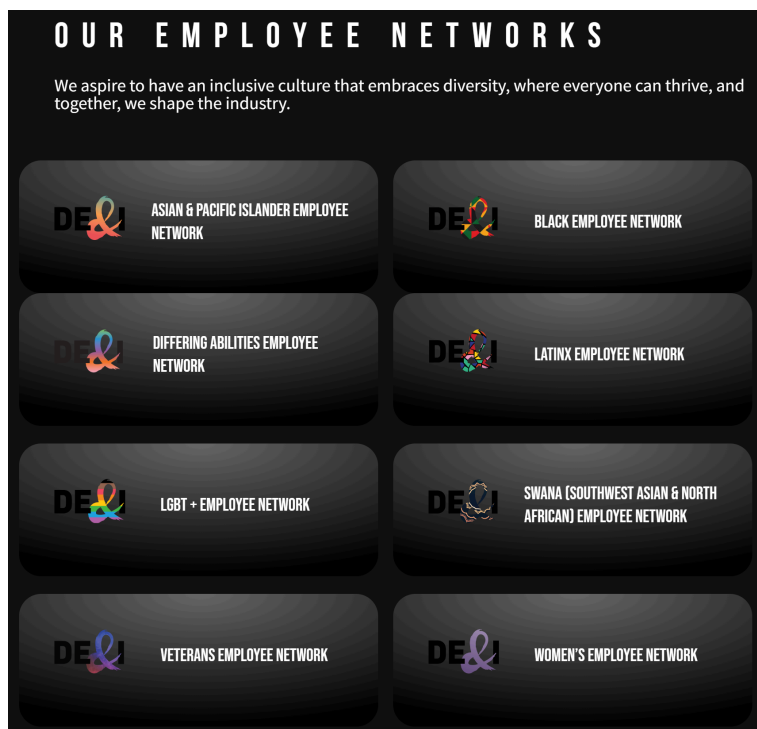
Discussion

A follow-up analysis of the above themes' prevalence is revealing. Companies such as Gearbox Software, Microsoft, and Blizzard Entertainment exceeded expectations for Theme 1 by providing multiple avenues to contact specific departments, including dedicated recruitment accessibility request forms from Microsoft and Blizzard.

Image 7.1. Gearbox Software, "Gearbox Software Contact Form"

Gearbox Software Contact Form

Email Recipient *

Careers/Internships

Please choose where you would like this message to be delivered.

Your Name *

Your Email Address *

Your Message Subject *

Your Message *

Making sure you're not an evil robot A.I.

I'm not a robot

reCAPTCHA
Privacy · Terms

SUBMIT

Image 7.2. Microsoft, "Accessibility request

Accessibility information

Please use this form to request any disability accommodation you might require during the application or recruiting process due to a disability. For example, an adjusted schedule, having documents read to you if necessary, using your preferred technology or using a sign language interpreter for the interview.

Rest assured that your request has been entered and a member of our support team will contact you within 48 hours.

Accessibility request

First name (required)

Last name (required)

Email address (required)

Description of disability accommodation request (required)

Please list the job ID's you would like to apply to

📄   Upload attachment/screenshot

Cancel   Submit

Image 7.3. Blizzard, "Accommodation Request"

Blizzard Entertainment is an Equal Opportunity Employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, gender expression, national origin, protected veteran status, or any other basis protected by applicable law, and will not be discriminated against on the basis of disability. Equal Employment Opportunity Policy.

Accommodation Request: We are committed to working with and providing reasonable assistance to individuals with physical and mental disabilities. If you are a disabled individual requiring an accommodation to apply for an open position, please email your request to accommodationrequests@activisionblizzard.com. General employment questions cannot be accepted or processed here. Thank you for your interest.

The majority of game companies where Theme 1 was present were dependent upon LinkedIn rather than more open channels of communication. By excluding LinkedIn, the original rate of 55% dropped to 30%. Further, the five game companies where only Theme 1 was present were all exclusively dependent upon LinkedIn, and would have otherwise had no themes present: Epic Games, Sony Santa Monica, BioWare, and Turn 10 Studios. Insomniac Games demonstrated an impressive degree of transparency regarding

the company recruiting process (Image 8.1) and excelled with Theme 1 by providing a dedicated contact form for Insomniac HR (Image 8.2), but failed to satisfy Theme 2 by not specifying any DEI-oriented values.

Image 8.1. Insomniac Games, recruiting process transparency

What If I don't see a job that fits my skill set? How often do you update your jobs?
Please keep an eye on our website for the role that does fit your skill set. We are always posting new opportunities, and look forward to hearing from you.

When will I hear from you about my application?
We review all submissions within 72 hours and reach out to qualified candidates within 7-10 days.

Do you offer internships?
Yes, we do! We post our internships in the fall for the upcoming spring/summer. Our interns are active participants in the creation of our games, and we look forward to this program each year. To be eligible, one could be an individual with limited experience 6-12 months in a chosen field, be a student (undergrad and grad), or a recent graduate (within 12 months of graduation). We view our internships as a fantastic way to launch into the creative games space.

How do I get into the video game industry?
While we cannot advise you on which school to attend or the classes to take, we do encourage you to go to school. Most of our artists have BFA's and our programmers have computer science or engineering degrees. Your school's academic advisers can suggest specific programs. While in college or a specialized game school, try to get an internship to gain on-the-job experience.

What should be on my demo reel or online portfolio?
We recommend only putting your best work on your demo reel or online portfolio. A concise reel is always appreciated – we will ask to see more work if we need to.

Does Insomniac offer remote work?
Yes- we do! We learned through the pandemic that great things can still happen – even when developers are not in the same office! We do require that you are eligible to work in the United States, live in the US, and are located in a state that Insomniac can support remote workers. If you have questions on this, as with any other question – please reach out via this form and we can clarify.

Image 8.2. Insomniac Games, "Contact Insomniac HR"

CONTACT INSOMNIAC HR

CAREERS

Name *

Email *

Enter Email　　　Confirm Email

How Can We Help? *

SUBMIT

FREQUENTLY ASKED QUESTIONS

A recruiter/HR person, claiming to be from Insomniac reached out to me, and asked me to download an app to apply. Is this real or a scam?
Any communication coming from Insomniac Games will come from the

However, said degree of transparency could be arguably more impactful than the typical diversity-speak (Kulik et al. 2021). This may demonstrate a partial flaw in this thematic structure and may motivate further research on DEI-oriented values in practice (i.e. Theme 3). Gearbox Software likewise provided significant transparency for the recruiting process (Image 9.1), but exceeded Insomniac by specifying DEI-oriented values. Gearbox Software also satisfied Theme 3 by referencing a history of fighting institutional transphobia (Image 9.2).

Image 9.1. Gearbox Software, recruiting process transparency

WHAT ARE THE STEPS IN THE PROCESS?

First you will apply to a position through our website then a recruiter and the hiring team will review your resume/portfolio. If you seem to be a good fit for the positon we may contact you to get some more information, ask you to complete a test, or have you send us some additional work samples. After we have all of the information we need we may schedule a phone interview and if that goes well you would be scheduled for an onsite interview. Typically the recruiter can follow up with you within a few days to let you know if you have been chosen for the position.

Image 9.2. Gearbox Software, "Standing Up for Our Team"

Standing Up for Our Team

We have a track record of standing up for our Gearbox family in the face of adversity. In 2017, Randy Pitchford joined other tech leaders in writing letters to Governor Abbott urging him to reject the 'bathroom bill' that would regulate bathroom use for transgender Texans. We also took a stand once again in 2021, fighting against bills in the Texas legislature that would inhibit voting rights and discriminate against transgender people. Not only do we fight for what is right within the walls of the Gearbox studio, we also actively work to keep Texas an attractive and equitable place for Gearbox artists, developers, storytellers, and professionals. As Randy Pitchford has said, our team at Gearbox "overwhelmingly supports non-discrimination protections and seeks to live in states that reflect the diversity and inclusion they value."

As for game companies where no themes were present, one of the two total stood out despite not adhering to the thematic structure. This paper perceived Respawn Entertainment as a company actively seeking talent – but there were only arguable claims of diversity with zero stated applications. Contrasted with its counterpart, 2K Games, Respawn Entertainment was perceived as more welcoming to prospective talent due to the game company website referencing said prospective talent. 2K Games, however, made no reference to developers whatsoever and was thus not perceived as a studio actively seeking talent at all – especially not disabled talent. Meanwhile, culture statements for companies such as Ubisoft expressed sentiments which satisfied Theme 2 and aligned with this paper's perspective on recruitment in the games industry: "Just as it takes years of iteration to develop a AAA title, we will only become a more diverse and inclusive company by continuously improving, trying new ideas and learning from the results" (Image 10.1). This statement was demonstrated in Ubisoft's transparent workforce representation statistics (Image 10.2), which satisfied Theme 3.

Image 10.1. Ubisoft, "Our Vision"

Our Vision

To create a video game, Ubisoft's teams around the world work together to push the boundaries of what's been done before and build an unforgettable experience for players. The journey from concept to launch requires collaboration, passion and commitment. We learn as we go, trying and failing and trying again before we get it right.

That same creativity, growth mindset and dedication form the foundation of our approach to diversity and inclusion. To truly have an impact, we must leverage innovative ideas and diverse perspectives to continue building a company – and an industry – that is welcoming to all.

Our vision of diversity and inclusion is multidimensional. That's why we've designed a strategy centered around four pillars: **colleagues, culture, content, and community.**

For Ubisoft, putting diversity and inclusion at the heart of everything we do means providing an environment where employees can thrive, building open-minded communities where players can connect, and creating games that reflect the diversity of the world we live in. We will not accomplish all these goals overnight. Just as it takes years of iteration to develop a AAA title, we will only become a more diverse and inclusive company by continuously improving, trying new ideas and learning from the results. It's a long-term commitment, one that we will tackle with the same passion and dedication that we bring to our games.
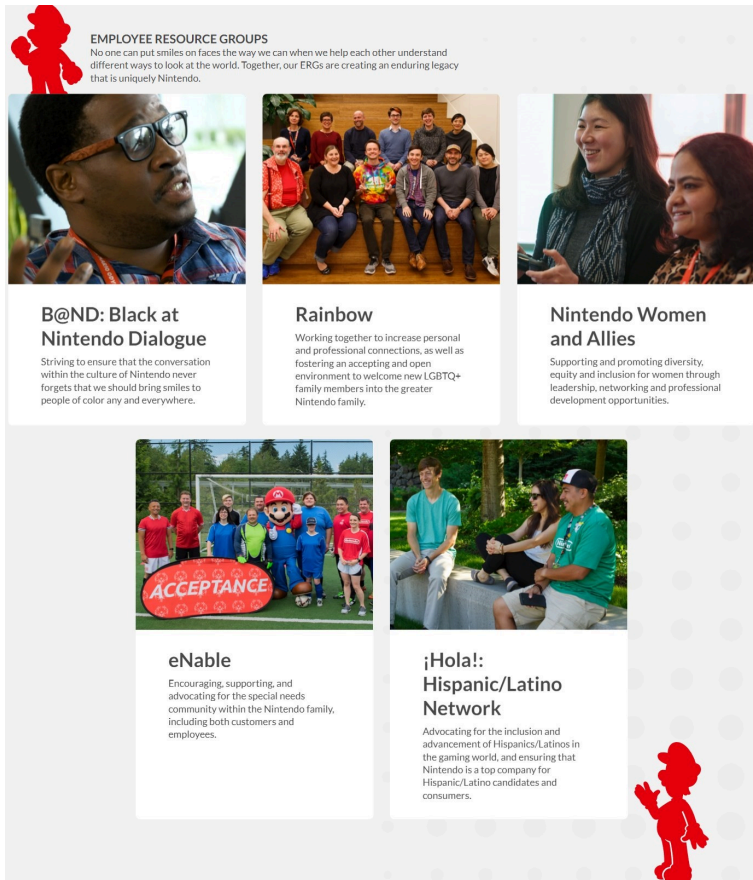
-Raashi Sikka, VP Global Diversity and Inclusion

Image 10.2. Ubisoft, workforce representation statistics

4068

Women in the Workforce

Increasing the number of women in our workforce is a key priority for Ubisoft. In March 2020, we set an objective to ensure women represent 24% of our workforce by March 2023. We are currently ahead of this target and will continue to aim higher moving forward.

113

Nationalities

Ubisoft is present in 31 countries, and the diversity of nationalities represented among our teams allows us to better understand our players and successfully adapt our games to a variety of cultural contexts.

32.9%

Percentage of new hires who were women

The percentage hire rate rose consistently over the last two years thanks to our teams' efforts to attract and recruit more diverse talent.

There were five game companies where Theme 4 was present exclusively due to disability or neurodiversity ERGs: Bungie, Wizards of the Coast, Electronic Arts, Ubisoft, and Nintendo of America. However, the language used to describe Nintendo of America's disability ERG raised alarming red flags, including even the ERG name: "eNable ERG. Encouraging, supporting, and advocating for the special needs community within the Nintendo family" (Image 11). The phrase "special needs" has been widely deprecated by the disabled community as an "ineffective euphemism" (Gernsbacher et al. 2016), and this paper therefore assumed minimal disabled involvement in Nintendo of America DEI initiatives.

Image 11. Nintendo of America, "eNable ERG"

As predicted, game companies acknowledging disabled GDs at all was significantly uncommon. The two game companies where only Theme 4 was not present were Gearbox Software and King – both of which performed well overall, but nonetheless failed to specifically address disability. Epic Games offered a comprehensive guide to company internships, and outright stated: "We wish to enable success for emerging talent by demystifying the critical talent pipelines and skills our teams look for and provide realistic pathways into Epic" (Image

12). And yet, Epic Games provided no evidence this mentality informed active recruitment strategies whatsoever.

Image 12. Epic Games, "When You Succeed, We Succeed"

When You Succeed, We Succeed

Our vision is to create the next generation of Epic talent. Exciting new opportunities will be open to those who have mastered the skills and technology that power gaming and interactive 3D content!
We wish to enable success for emerging talent by demystifying the critical talent pipelines and skills our teams look for and provide realistic pathways into Epic through early career opportunities.

Anyone can benefit from the free tools that are available to help hone their skills and build immersive new worlds. Be a part of the next generation at Epic and get started with learning resources today through Unreal Online Learning.

Theme 3 was instead present for Riot Games, and DEI-oriented values were consistent for a variety of marginalized identities and corresponding ERGs (Image 13).

Image 13. Riot Games, ERGs

API@Riot

Foster a place for Rioters of all Asian & Pacific Islander identities to come together, learn from each other's shared commonalities and multicultural differences, and amplify the voices of the global API community at the Riot level.

Filipinos At Riot

Filipinos At Riot's (FAR) mission is to empower, unify, and celebrate Filipinos at Riot and in the gaming community.

Rainbow Rioters

Rainbow Rioters' mission is to foster LGBTQIA+ inclusive communities and products at Riot and beyond.

Riot Alliance of Diverse Genders

Riot Alliance of Diverse Genders' (RAD Genders) mission is to drive Riot to be a place where people of all genders thrive and see themselves reflected in Riot's products.

Riot Noir

Riot Noir's mission is to amplify Black voices and increase representation at Riot both in and outside of products.

Riot Unidos

Riot Unidos' mission is to provide a supportive environment for Latinx Rioters and their allies by advocating for increased visibility, cultural bonding, community outreach, and positive change throughout Riot Games.

Veterans@Riot

Veterans@Riot's mission is to support Veteran Rioters and allies while increasing representation in gaming and entertainment.

Electronic Arts also satisfied Theme 3, and stood out by highlighting pay equity (Image 14.1) and user accessibility (Image 14.2). Even still, Riot, Epic, and Electronic Arts were all alike in seeming to forget disabled developers altogether.

Image 14.1. Electronic Arts, "Our Commitment to Pay Equity"

Our Commitment to Pay Equity
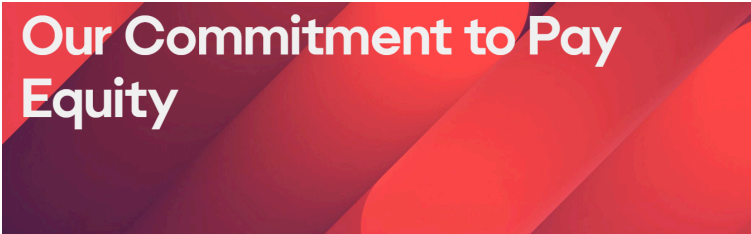
As part of our commitment to building more diverse and healthy teams, we're constantly looking at how we can embed principles and practices of inclusion across our systems, processes and culture.

Part of this commitment focuses on compensating our employees fairly based on the work that they do. It also means being transparent about our approach and being accountable for it, which is why we're choosing to share our pay equity information for the second consecutive year.

Ensuring we're as consistent as we are fair in our approach to compensation is a priority at every stage of the compensation process, from the moment we hire, to annual raises, to promotions. We analyze compensation during our annual review cycles and when promoting employees. In addition, we annually partner with an independent outside firm to review employees' pay which promotes fairness and reduces the risk of unconscious bias in our compensation philosophy and practices. When we find compensation that deviates from what we would expect to see based on our practices, we take action.

Image 14.2. Electronic Arts, "Accessibility Portal"

Accessibility Portal

Over a billion people in the world have a disability. EA's Accessibility Team aims to lessen the gap between capability and disability by breaking down barriers that prevent play. We support efforts within EA to make our games and services more accessible to players of all abilities and believe that each one of us has the power to make a difference in the lives of others.

If you run into any issue with any EA site or product, please file a bug using this Bug Report forum. If you have any technical help questions, please use the Technical Issues forum.

At EA we strive to bridge ideas and innovations that aid accessibility company and industry wide. To learn more about EA's Patent Pledge for Increasing Accessibility, click here. For additional information, please visit ea.com/news blog here.

The only three game companies that provided recruiter info, specified and provided examples of DEI-oriented values, and included disability in those examples, were: Activision, Ubisoft, and Microsoft. And even among these, disability was spottily considered. The description of Ubisoft's Neurodiversity ERG was noticeably less people-focused than other Ubisoft ERGs (Image 15).

Image 15. Ubisoft, ERGs

A.P.I (Asian & Pacific Islanders)

The Asian & Pacific Islander (API) ERG advocates for the diverse needs of API within Ubisoft, including but not limited to: Inclusion and awareness, career advancement, professional development, and community outreach and volunteer efforts.

B.E.A.U. (Black Employees at Ubisoft)

Black Employees at Ubisoft (B.E.A.U.) is to foster community, to improve representation, cultural awareness and competency and to champion the advancement of Black employees at Ubisoft to create a more diverse and inclusive workplace.

GENTE (Latine/Hispanic)

Gente recognizes the diversity of the Latine community, as well as its rich cultural history rooted in community and family. As an ERG, its mission is to unite gente at Ubisoft and champion the community culturally, professionally, and creatively as a collective voice.

Salaam (Middle East & North Africa)

Salaam's mission is to empower the Middle East and North African identity within the video-game industry through the career development of Ubisoft employees and subsequent support of the greater M.E.N.A community to improve cultural competency in the industry.

Neurodiversity

The Neurodiversity ERG is working to serve as a reference group on Neurodiversity for all Ubisoft employees, providing safe spaces for team members to exchange experiences. Its focus is on raising awareness, supporting inclusion initiatives at work and ensuring video games foster belonging for all.

UbiProud (LGBTQIA2S+)

UbiProud is a community of queer people across Ubisoft worldwide. Its members want to create a safe space for all and help each other excel at the workplace and provide the opportunities to do so. UbiProud advocates for equality, inclusion, and representation within our games, offices and studios.

Black Employees at Ubisoft was described as "to champion the advancement of Black employees." UbiProud, a queer ERG, was described as "members want to… help each other excel at the workplace and provide the opportunities to do so." Then, the Neurodiversity ERG stated, "its focus is on… ensuring video games foster belonging for all." Activision's Differing Employee Network ERG also emphasized disabled users over disabled GDs (Image 16). Nintendo of America's aforementioned ERG exhibited a similar discrepancy between a disability ERG and other ERGs (Image 11).

Image 16. Activision, "Accessibility in Our Games"

ACCESSIBILITY IN OUR GAMES

At Activision, we strive to make great games – for all. Our passion for inclusion extends to the craft and care we put into making our games accessible. Through a variety of accessibility features - such as robust subtitles, flexible color vision deficiency filters, and bespoke controller options - we aim to welcome all players to enjoy the epic gaming experiences our company has to offer.

Microsoft seemed to stand alone as the most comprehensive and accessible game company website. Where Insomniac surpassed typical diversity-speak, Microsoft surpassed Insomniac due to consistent and constant multimodal communication to candidates (Image 17.1). Inclusive design was specified as a primary informant of company values (Image 17.2), and its application was apparent in the game company website itself.

Image 17.1. Microsoft, "Inclusive interviewing"

Inclusive interviewing

At Microsoft, we provide more than accommodations, we host inclusive interviews to make sure candidates are set up for success. By providing an interview process that offers accommodations upfront, such as less interviews in a day, longer breaks between interviews and a 1/1 interactive discussion on accommodations we help remove barriers to the interview process and create a positive interviewing environment for candidates with disabilities.

Watch video ›

Image 17.2. Microsoft, "Inclusive thinking drives our innovation"

Inclusive thinking drives our innovation

We design for all human experiences and needs. So, we strive to gather, listen to, and include as many perspectives as possible, with the goal of discovering how to bring out the best in each other, and everyone who uses what we create.

Our inclusive design principles >

Even still, Microsoft provided extensive examples: "Microsoft is dedicated to infusing diversity and inclusion principles into our hiring, our communication, our innovation" (Image 17.3).

Image 17.3. Microsoft, "We're taking action"

We're taking action

From our leaders to the larger community, Microsoft is dedicated to infusing diversity and inclusion principles into our hiring, our communication, our innovation, and the way we build products and technologies.

Between transparency regarding workforce diversity (Image 17.4), pay equity (Image 17.5), diversity and inclusion training (Image 17.6), and inclusive hiring programs (Image 17.7) – Microsoft seemed to almost pass with flying colors. So, why did these exciting findings still feel unsatisfying?

Image 17.4. Microsoft, workforce representation statistics

+20.2%

6.6%

Global women

Globally the employee population of women grew 20.2% in 2022 and has grown 81.3% since 2018. We've increased the number of women at Partner + Executive (+102.2%) and Director levels (+112.3%) since 2018.

Read more >

US Black and African American

Black and African American representation in our core US workforce rose 0.9 percentage points to 6.6%, the highest year-over-year increase in the past five years.

Read more >

7.6%

85.1%

US Hispanic and Latinx

Hispanic and Latinx representation in our core US workforce rose 0.6 percentage points to 7.6%, the highest year-over-year increase in the past five years.

Read more >

Diverse and inclusive

85.1% of employees agreed or strongly agreed that "we're diverse and inclusive ... we're open to others' ideas, we value and invite differing perspectives, and we believe diversity is critical to our success," up 1.2 percentage points.

Read more >

Image 17.5. Microsoft, pay equity

$1.008

$1.007

$1.002

Racial and ethnic minorities in the US

As of September 2022, inside the US, racial and ethnic minority employees combined earn $1.008 total pay for every $1.000 earned by white employees with the same job title and level and considering tenure.

Women in the US

As of September 2022, inside the US, women employees earn $1.007 total pay for every $1.000 earned by employees who are men and have the same job title and level and considering tenure.

Women outside of the US

As of September 2022, outside the US, women employees earn $1.002 total pay for every $1.000 earned by employees who are men with the same job title and level and considering tenure in these combined geographies.

Image 17.6. Microsoft, diversity and inclusion training

Together, we learn and grow

At Microsoft we're looking to sociology, psychology, behavioral science and neuroscience to understand what leads to exclusion, and to find effective ways to change our habits and behaviors. We gladly share our learning resources so that others can create inclusive environments where all people feel valued, heard and included.

Explore the full library of content >

Image 17.7. Microsoft, inclusive hiring programs

Inclusive hiring for people with disabilities

From the very first days of our company, Microsoft has sought to enable
individuals and organizations around world to do great things.

Neurodiversity program
Recruit, onboard and development of
Neurodiverse individuals.

To apply or learn more ›

Supported employment
Supporting people with intellectual
developmental disabilities via Real
Estate & Facilities vendor ecosystem.

Learn more ›

Disability hiring
Every day at Microsoft, we are hiring
people with disabilities into full time
roles across the company.

To apply or learn more ›

Conclusion

Where other marginalized identities are frequently accounted for, disabled GDs are widely disregarded. At best, internal retention resources like ERGs, or inclusive hiring programs, are available. At worst, game companies seem to perceive disabled people only as players. Microsoft was a beacon of hope as one of the final game companies examined in this study. Soon after, however, I was annoyed to be impressed by the sole game company that even approached the bare minimum for digital access. My initial excitement for Microsoft's success diminished quickly when I found no evidence to suggest inclusive hiring programs are anything more than alternative job streams contingent upon self-disclosure of disability, and are potentially more vulnerable to prejudice compared to a unified job stream with inclusive policies meant to assist universal applicants.

Furthermore, ERGs and other internal retention resources only support current employees. By locking the vast majority of demonstrably limited assistance for disabled developers behind closed doors, game companies reveal their bias and support this paper's hypothesis that games recruitment is incorrectly assumed to be a neutral or objective practice. How

could game companies consider disabled people as candidates for the next generation of the games industry when they seemingly fail to support or acknowledge the 25% of disabled developers populating studios today (IGDA 2019)?

Notably, a growing number of game companies continue to rescind early COVID era work-from-home accommodations. It is this paper's stance that such policy shifts are predicated in ableism. That said, it is worth distinguishing that office-oriented policies are not evil and work-from-home policies are not unilaterally good; disability, and by extension accessibility, is not a monolith. Rather, forced return-to-office policies are ableist because they restrict developer agency to a disabling degree. As a consequence, more and more game companies will outright disqualify disabled developers from honing their craft as future talent recruitment – and simultaneously endanger the health and livelihoods of disabled talent in the industry today.

Despite disabled candidates nonetheless seeking entry into professional game development, there are few reasons to suspect disability is accommodated or even allowed in AAA contexts. This paper recommends that game companies with inclusive values and companies desiring disabled prospective talent provide more explicit examples of ongoing EDI initiatives in public facing documentation.

Further research may better illuminate the impacts of inclusive hiring programs on disabled candidates in the games industry. Further research is also needed to identify industry norms negatively affecting recruitment and retention of disabled GDs.

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References

Ameri, M., Schur, L., Adya, M., Bentley, F. S., McKay, P., & Kruse, D. (2018). The Disability Employment Puzzle: A Field Experiment on Employer Hiring Behavior. ILR Review, 71(2), 329–364. https://doi.org/10.1177/0019793917717474

Andersson, J., Luthra, R., Hurtig, P., et al. (2015). Employer attitudes toward hiring persons with disabilities: a vignette study in Sweden. Journal of Vocational Rehabilitation, 43(1), 41–50. Retrieved from https://content.iospress.com/articles/journal-of-vocational-rehabilitation/jvr753.

Baert, S. (2016). Wage subsidies and hiring chances for the disabled: some casual evidence. Eur J Health Econ, 17, 71–86. https://doi.org/10.1007/s10198-014-0656-7

Charmaz, K. (2010), "Disclosing illness and disability in the workplace", Journal of International Education in Business, Vol. 3 No. 1/2, pp. 6-19. https://doi.org/10.1108/18363261011106858

Cote, Harris, B. C. (2021). The cruel optimism of "good crunch": How game industry discourses perpetuate unsustainable labor practices. New Media & Society. https://doi.org/10.1177/14614448211014213

Cote, & Harris, B. C. (2020). 'Weekends became something other people did': Understanding and intervening in the habitus of video game crunch. Convergence (London, England), 27(1), 161–176. https://doi.org/10.1177/1354856520913865

Das, M., Tang, J., Ringland, K., Piper, A. M. (2021). Toward Accessible Remote Work: Understanding Work-from-Home Practices of Neurodivergent Professionals. Association for Computing Machinery, 5(183), 1–30. https://doi.org/10.1145/3449282

Dyer-Witheford, N., de Peuter, G. (2006). "EA Spouse" and

the Crisis of Video Game Labour: Enjoyment, Exclusion, Exploitation, Exodus. Canadian Journal of Communication, 31(3), 599–617. https://doi.org/10.22230/cjc.2006v31n3a1771

Flanagan, M. (2009). Critical Play : Radical Game Design. MIT Press. Retrieved from https://utah-primoprod.hosted.exlibrisgroup.com/permalink/f/dtufc4/UUU_ALMA51422 706070002001.

Fougeyrollas, P., Boucher, N., Edwards, G., Grenier, Y. and Noreau, L., 2019. The Disability Creation Process Model: A Comprehensive Explanation of Disabling Situations as a Guide to Developing Policy and Service Programs. Scandinavian Journal of Disability Research, 21(1), pp.25–37. http://doi.org/10.16993/sjdr.62

Gernsbacher, M. A., Raimond, A. R., Balinghasay, M. T., & Boston, J. S. (2016). "Special needs" is an ineffective euphemism. Cognitive research: principles and implications, 1(1), 29. https://doi.org/10.1186/s41235-016-0025-4

Hahn, H. (1985). Toward a Politics of Disability: Definitions, Disciplines, and Policies. University of Southern California. Retrieved from www.independentliving.org/docs4/hahn2.html.

Hamonet, C., Gracies, J.-M. (2013). Disabling situations, an original concept connecting disease, disability, and rehabilitation to assess and manage disabled persons. 7th World Congress of the International Society of Physical and Rehabilitation Medicine. Retrieved from http://claude.hamonet.free.fr/eng/art_disability-situations.htm

Hoque, K., Bacon, N. (2021). Working from home and disabled people's employment outcomes. British Journal of Industrial Relations, 60(1), 1–251. https://doi.org/10.1111/bjir.12645

Kattari, S. K., Olzman, M., & Hanna, M. D. (2018). "You Look Fine!": Ableist Experiences by People With Invisible

Disabilities. Affilia, 33(4), 477–492. https://doi.org/10.1177/0886109918778073

Keller, R. M., & Galgay, C. E. (2010). Microaggressive experiences of people with disabilities. In: D. W. Sue (Ed.), Microaggressions and marginality: Manifestation, dynamics, and impact, 241–267. John Wiley & Sons Inc.

Kulik, J., Beeston, J., Cairns, P. (2021). Grounded Theory of Accessible Game Development. Association for Computing Machinery, 28, 1–9. https://doi.org/10.1145/3472538.3472567

Kwon, C., Archer, M. (2022). Conceptualizing the Marginalization Experiences of People with Disabilities in Organizations Using an Ableism Lens. Human Resource Development Review. https://doi.org/10.1177/15344843221106561

McLaughlin, M. E., Bell, M. P., Stringer, D. Y. (2004). Stigma and Acceptance of Persons With Disabilities: Understudied Aspects of Workforce Diversity.

Mik-Meyer, N. (2016). Othering, ableism and disability: A discursive analysis of co-workers' construction of colleagues with visible impairments. Human Relations, 69(6), 1341–1363. https://doi.org/10.1177/0018726715618454

Niemelä, J. (2021). A Systematic Mapping Study of Crunch Time in Video Game Development. University of Oulu. Retrieved from https://oatd.org/oatd/record?record=oai\:oulu.fi\:nbnfioulu-202106178507.

O'Donnell. (2014). Developer's Dilemma : the secret world of videogame creators. Retrieved from https://utah-primoprod.hosted.exlibrisgroup.com/permalink/f/dtufc4/UUU_ALMA51559 730820002001.

Reber, L., Kreschmer, J. M., James, T. G., Junior, J. D., DeShong, G. L., Parker, S., & Meade, M. A. (2022). Ableism and Contours of the Attitudinal Environment as Identified by

Adults with Long-Term Physical Disabilities: A Qualitative Study. International Journal of Environmental Research and Public Health, 19(12), 7469. MDPI AG. http://dx.doi.org/10.3390/ijerph19127469

Revillard, A. (2022). Disabled People Working in the Disability Sector: Occupational Segregation or Personal Fulfillment? Work, Employment and Society. https://doi.org/10.1177/09500170221080401

Scholz, F. (2020). Taken for Granted: Ableist Norms Embedded in the Design of Online Recruitment Practices. In: Fielden, S.L., Moore, M.E., Bend, G.L. (eds) The Palgrave Handbook of Disability at Work. Palgrave Macmillan, Cham. https://doi.org/10.1007/978-3-030-42966-9_26

Syma, C. (2019), "Invisible disabilities: perceptions and barriers to reasonable accommodations in the workplace", Library Management, Vol. 40 No. 1/2, 113-120. https://doi.org/10.1108/LM-10-2017-0101

Taylor, T. L. (2006). Play Between Worlds: Exploring Online Game Culture. Habitat LucasArts Inc, MIT Press.

Walls, R.T., Batiste, L.C. (1996). Job Accommodations for Fatigue in the Workplace. Technology and Disability, Vol. 5 No. 3–4, 334–343.

Weststar & Legault, M.-J. (2019). Building Momentum for Collectivity in the Digital Game Community, Television & New Media, 20(8), 848–861. https://doi.org/10.1177%2F1527476419851087

Weststar, J., Kumar, S., Coppins, T., Kwan, E., Inceefe, E. (2021). Developer Satisfaction Survey 2021. International Game Developers Association. Retrieved from https://igda-website.s3.us-east-2.amazonaws.com/wp-content/uploads/2021/10/18113901 /IGDA-DSS-2021_SummaryReport_2021.pdf.

Weststar, J., Kwan, E., Kumar, S. (2019). Developer

Satisfaction Survey 2019. International Game Developers Association. Retrieved from https://s3-us-east-2.amazonaws.com/igda-website/wp-content/uploads/2020/01/29093706 /IGDA-DSS-2019_Summary-Report_Nov-20-2019.pdf.

Weststar, & Legault, M.-J. (2017). Why Might a Videogame Developer Join a Union? Labor Studies Journal, 42(4), 295–321. https://doi.org/10.1177/0160449X17731878

Østerud, K. L. (2022). Disability Discrimination: Employer Considerations of Disabled Jobseekers in Light of the Ideal Worker. Work, Employment and Society. https://doi.org/10.1177/09500170211041303

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24. **Research Reflection by Will Loxley**

Will Loxley

Faculty Mentors: Fernando Rodríguez and Ashley Guajardo (Entertainment Arts Engineering, University of Utah)

When I first approached my mentors Dr. Guajardo and Dr. Rodríguez with the idea to investigate ableism in games recruitment, I was pleasantly surprised to find they were as eager to begin as I was. Before my experience with this project, I was not familiar with the practical processes involved in research. Now, thanks to my mentors and nine months of research, I am confident in my abilities to identify necessary methodologies and utilize them to execute on a point of interest. And after presenting my findings at the 2023 Utah Conference on Undergraduate Research, I am prepared to adequately communicate data and conclusions to a broad audience. As a prospective game developer and disability advocate, my overarching career goal is to enable marginalized

creatives in a dynamic and ever-changing industry. I believe the games we make are decisively better when made with a wider pool of perspectives, and that broadening the definition of the "average game developer" will be vital for the long-term success of the games industry. I want to be engaged in work that not only carves out space for disabled developers in the industry, but also contributes to smoothing out the transition from student to professional. This research has given me the opportunity to start that pursuit, and I'm grateful that this is only the beginning.

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25. **Temperature Dependence of Leptospirillum Ferriphilum During Biooxidation of Pyrite**

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Faculty Mentor: Michael Free (Materials Science and Engineering, University of Utah)

ABSTRACT

Rare earth elements have properties that make them useful in advanced electronics, magnets, and batteries. However, they are difficult to isolate from their constituent elements, which results in an environmentally costly operation to refine them. An environmentally friendly alternative extraction method involves biooxidation which uses bacteria to generate acid and ferric ions from pyrite to free the REEs from chemically bonded constituents. Because bacteria are living creatures, one of the most important factors to consider when running a bioreactor is the operating temperature. This study involved testing and analysis of the temperature dependence of biooxidation using pyrite concentrated from coal waste. The temperature was varied from 25℃ to 40℃. Acid production in the bioreactor was monitored with pH measurements and bacterial oxidation was measured using the oxidation-reduction potential (ORP) of the system. Bacterial vitality was monitored by periodic ferrous oxidation tests which quantitatively assessed the biooxidation rate. The ferrous biooxidation rate (BOR) was evaluated using the Michaelis- Menten or Monod kinetic equations. Elemental and volumetric mass balances were done after each parameter. Pyrite recoveries were analyzed using energy-dispersive x-ray spectroscopy (EDS), scanning electron microscope (SEM) analysis, and x-ray diffraction (XRD). Additionally, bacteria species analysis showed that Leptospirillum ferriphilum was the dominant bacteria species, showing divergence from the original Acidithiobacillus ferrooxidaans culture. Analysis showed that 35℃ had the lowest pH, highest ORP, and highest BOR, while 40℃ caused bacterial death.

INTRODUCTION

Rare earth elements (REEs), such as yttrium and lanthanides

are widely used in technology and engineering as vital components of cell phones, electronics, and magnets [1]. Contrary to their name, REEs are relatively abundant on the earth's crust. Previous studies have found that particularly concentrated samples of REEs in coal-mining deposits, which this study focuses on [2]. However, REEs are difficult to refine and extract because they naturally occur with constituent elements [3]. It is, therefore, vital that alternative extraction techniques be explored to sustain the growing demand for REEs while compensating for the decrease in their natural abundance [3].

Solvent extraction is the primary industrial extraction method used to separate REEs from solutions obtained from leaching appropriate ores. Activated carbon and polymeric resin have been explored as a possible alternative to solvent extraction but has not been implemented due to low yield [1]. Solvent extraction in particular uses hazardous chemicals that pose an environmental threat. Traditional solvents, like tributyl phosphate, di-(2-ethylhexyl)phosphoric acid, and Cyanex 272, are effective but toxic and difficult to dispose. Attempts have been made to replace these solvents with benzene, heptane, kerosene, and in some cases, ionic liquids. But many of these replacements show poorer results than traditional solvents and have not been accepted for commercial use [1].

The extraction of REEs using biooxidation has been the subject of vigorous research in the last decades. Biooxidation is the process of using bacteria to free the desired elements from chemically bonded constituents. Since REEs rarely occur in elemental form, biooxidation is easily applicable to the extraction of these metals. This method is particularly appealing because it provides a low-cost, relatively efficient, and non-polluting extraction option [1] [4] [5].

Biooxidation using coal-mining deposits would provide an environmentally friendly extraction method, while also increasing U.S. domestic REE production. A variety of microbes are used in biooxidation however, regardless of the species, these bacteria oxidize the ferrous ions ($Fe2^+$) released from pyrite to ferric ions ($Fe3^+$). The resulting ferric ions oxidize pyrite and produce sulfuric acid as a byproduct. The bacteria that catalyze the pyrite leaching used in this experiment use a combination of biotic and abiotic reactions:

$$14Fe^{2+} \rightleftharpoons 14Fe^{3+} + 4e^- \quad \text{(biotic)}$$

with:

$$\frac{7}{2}O_2 + 14H^+ + 14e^- \rightleftharpoons 7H_2O \quad \text{(biotic)}$$

and:

$$14Fe^{3+} + 8H_2O + FeS_2 \rightleftharpoons 15Fe^{2+}16H^+ + 2SO_4^{2-} \quad \text{(abiotic)}$$

combine, producing sulfates (SO_4^{2-}), and acid (H^+) as by-products, as seen in the overall reaction:

$$FeS_2 + \frac{7}{2}O_2 + H_2O \rightleftharpoons Fe^{2+}2SO_4^{2-} + 2H^+$$
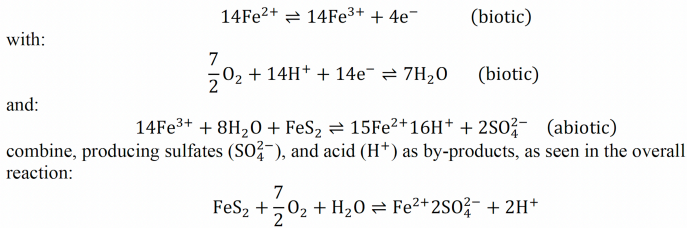
The biooxidation rate is a crucial parameter that measures the bacteria vitality in the bioreactor. It can be measured using the Nernst equation (1), (2), and Michaelis-Menten kinetics (3):

$$E = E_o - \frac{RT}{nF} \ln \frac{a_{Fe^{2+}}}{a_{Fe^{3+}}}$$

(1)

The electrochemical potential is 'E', the standard potential is 'Eo', the gas constant is 'R', the absolute temperature is 'T', the

number of electrons is 'n', the activity is 'a', and the Faraday constant is 'F'. This equation can be rearranged as:

$$E = E_o + \frac{RT}{nF} \ln \frac{\gamma_{Fe^{3+}} m_{Fe^{3+}}}{\gamma_{Fe^{2+}} m_{Fe^{2+}}}$$

(2)

The initial concentration of ferrous ions can be determined using Michaelis-Menten kinetics. Michaelis-Menten kinetics calculate the rate of biooxidation using the initial (3) and final (4) ferrous ion concentrations.

$$m_{Fe^{2+}_{init}} = \frac{m_{Fe^{2+}_{added}}}{\exp\frac{(E_{init} - E_{afterFe2+addition})nF}{RT} - 1}$$

(3)

which can be rearranged using (2) to obtain:

$$m_{Fe^{3+}_{init}} = \exp\frac{(E_{init} - E_o)nF}{RT} \frac{m_{Fe^{2+}_{init}} \gamma_{Fe^{2+}}}{\gamma_{Fe^{3+}}}$$

(4)

If Eo is assumed to be 0.77 V, the oxidation rate can be expressed as:

$$R_{Fe^{2+}} = \frac{C_{cells} \mu_{max} C_{Fe^{2+}}}{Y_c(C_{Fe^{2+}} + K_m)}$$

(8)

where Yc is the cell yield coefficient, Km is the Michaelis constant, Ccells is the cell concentration and μmax is the maximum specific growth rate. The Michaelis constant was calculated using the Lineweaver-Burke plot.

$$\frac{1}{R_{Fe^{2+}ox}} = \frac{Y_c K_m}{\mu_{max} C_{cells} C_{Fe^{2+}}} + \frac{Y_c}{\mu_{max} C_{cells}} \qquad (9)$$

In this study, the rate of biooxidation was tracked by daily measurements of the oxidationreduction potential (ORP), which was reported as the ORP or the electrochemical potential of a redox electrode. Bacterial vitality was monitored by periodic ferrous oxidation testing to quantify the bacterial population by adding a predetermined quantity of ferrous sulfate and subsequent monitoring ferrous ion oxidation. From this data, the level of the bioreactor, the amount of ferrous sulfate added, the temperature of the bioreactor, and ionic strength, the average ferrous oxidation rate can be calculated using a modified Nernst equation (2).

Biooxidizing bacteria operate in extremely low pH (1-2), moderately high temperature, and oxygenated environments [6] [7]. Previous studies have attempted to optimize pH, and oxygen concentration operating parameters for Acidithiobacillus ferrooxidans as well as related microbes such as Thiobacillus ferrooxidans and Leptospirillum ferriphilum [8] [9] [10]. While past studies have presented optimal temperature ranges for biooxidation, there are few conclusive studies that identify the exact optimal temperature for REE extraction [10] [11] [12]. This study investigates the effect of temperature on biooxidation rate, pH, ORP using a biooxidizing bacteria. This study also presents a bacterial species assay of the composition of bacteria within the bioreactor. The results of this study are analyzed using scanning electron microscope (SEM), energy-dispersive spectroscopy (EDS), x-ray diffraction (XRD), and inductively coupled plasma mass spectrometry (ICP-MS) analysis.

METHODS

Set-up A 3L Chemglass bioreactor is attached to a feeding flask and a collection flask. The feeding flask is filled with the feeding solution which varies with each parameter but consistently contains DI water, a slurry of 9K nutrients, and refined pyrite (Figure 1). An overhead stirrer, which is controlled by an automatic timer, is inserted into the feeding flask, and turns on 5 minutes before the scheduled feeding time to ensure that a homogenous solution of liquid and pyrite is pumped into the bioreactor. The feeding slurry is pumped into the bioreactor while solution from the bioreactor is collected and pumped into the collection or effluent flask.

Figure 1: The bioreactor (left) is fed via a feeding flask (right) which contains pyrite, a 9K medium and DI water.
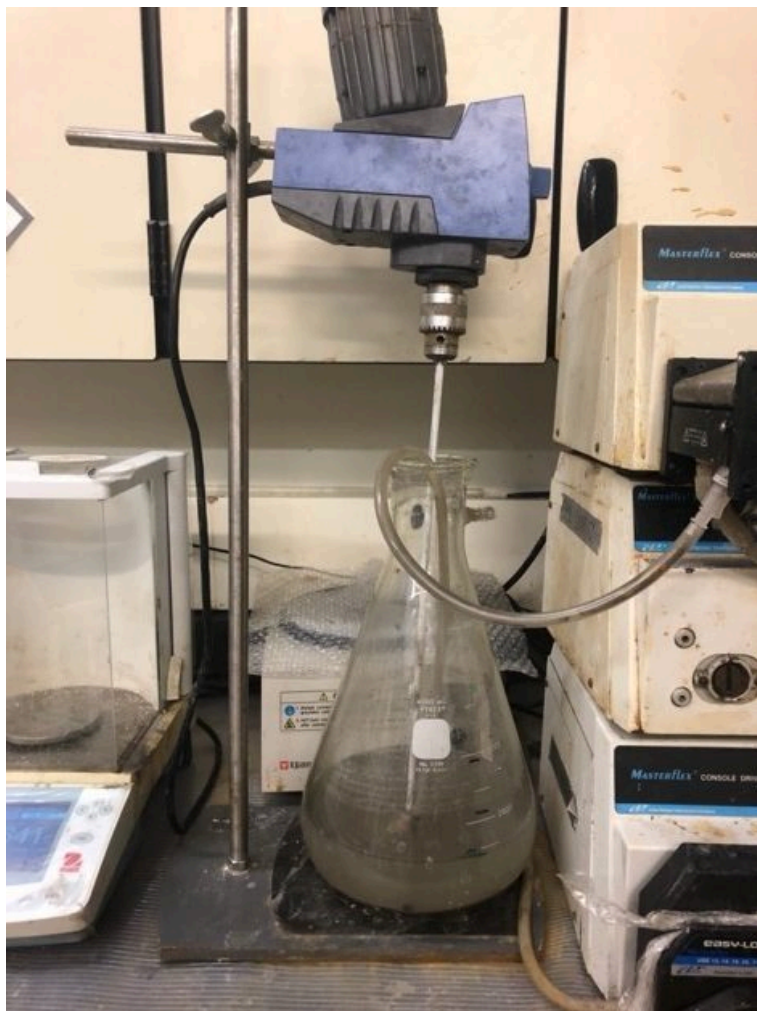
Since the bacteria require oxygen, pressurized air is pumped into the bioreactor through an automatic airflow meter. The bioreactor is kept at a constant temperature by a water bath, where water is heated in the bath and pumped into a closed port of the bioreactor and back into the bath. Thus, the heated water never mixes with the bioleaching solution. The bioreactor is agitated by a ChemGlass overhead stirrer and

motor, whose speed is adjustable. Because the instruments that are attached to the lid of the bioreactor raise the level slightly, the volume of the bioreactor used to calculate the average ferrous oxidation rate must be adjusted. In this experiment, if the volume was 2600mL in the bioreactor it was recorded as 2400 mL to account for this difference.

As seen in Table 1, the temperature is varied as residence time, solid weight percentage, and airflow were held constant.

A steady state was established in the bioreactor before data was collected. Once the reactor was operated for approximately two residence times and steady readings for 3 consecutive days were recorded, the data was recorded and treated as representative of the bioreactor's behavior. The data were averaged over the span of the mass balance.

Acid Generation:

The acid generation of the bioreactor is of particular importance in controlling the homogeneity of the solution. It has been shown in previous studies that predominately jarosite precipitates will form at pH~2.3. These precipitates can form in the bioreactor which inhibits the mechanical ability of the bioreactor and interferes with the bacteria's ability to oxidize the pyrite. A low pH shows that the bacteria are generating their own acid. It was, therefore, essential to keep the pH below 2.3. If the pH rose above 2.3, it was corrected by adding a small amount of dilute sulfuric acid. The acid production of the bioreactor was monitored through daily pH readings.

ORP and Biooxidation Rate:

The ORP and biooxidation rate (BOR) of the reactor give essential information about the vitality and activity of the bacteria. The ORP and BOR are excellent indicators of mechanical problems inside the bioreactors as they are very

sensitive to changes in RPM, airflow, and temperature. For this experiment, only the temperature was varied.

In the context of this experiment, ORP is analogous to the appetite of the bacteria and BOR to the speed at which the bacteria eat. The BOR was calculated by introducing 1g of FeSO4·7H2O into the bioreactor. The ORP probe was then submerged into the bioreactor. The ORP reading dropped sharply with the introduction of the ferrous sulfate. Once the ORP began to increase, the time for the ferrous oxidation test started. ORP readings were taken every 30 seconds until it reaches within 4-6mV of its starting value. As stated above, the BOR was calculated using the modified Nernst equation (2).

Mass Balance:

Two types of mass balance were performed during this experiment: overall and element-based mass balance. The overall mass balance was calculated after the completion of each parameter by measuring the weight of the effluent solution and comparing it with the mass of the added solution and remaining solution. An average evaporation rate of 7.63% per day was used to calculate the percent yield and determine the efficiency of feed and collection.

For element-based mass balance, effluent and solid samples were analyzed using ICP-MS. ICPMS was done separately on the solid and liquid samples from each steady-state parameter. For a complete picture of the mass balance, the mass balance data were combined in the results. Effluent and solid samples from each steady-state parameter were also analyzed using EDS, SEM and XRD analysis. Before samples went into the XRD machine, they were crushed with a mortar and pestle.

RESULTS

Figure 2 shows the raw data from a sample biooxidation test taken at 35℃. The addition of ferrous sulfate to the bioreactor

causes a sharp increase in the presence of ferrous ions in the bioreactor solution as seen in Figure 2a. The biooxidation test starts at time = 0 when the concentration has reached its maximum. As the test goes on, the ferrous ions are consumed by the bacteria and consequently, their concentration decreases to 0. This data is analyzed with Michaelis-Menten kinetics to produce Figure 2b.

Figure 2: Example of data collected during a ferrous ion biooxidation test
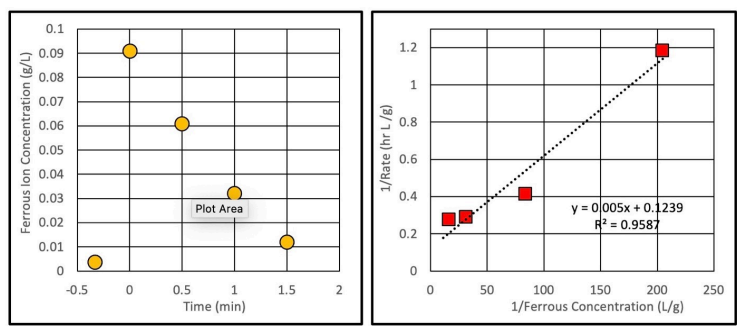
Figure 2: Example of data collected during a ferrous ion biooxidation test

As the temperature increased there were three noticeable trends in the collected data as illustrated in Figure 3. The increase in temperature from 25℃ to 35℃ caused the average pH to decrease, the average ORP to increase, and the BOR to increase. Figure 4 shows the precipitous decline in BOR recorded during 40℃. There is an initial increase in the BOR from 1.3 7 8∗:; to 2.5 7 8∗:; over the course of four days. This is followed by a drop to 0 7 8∗:; during the next week.

Figure 3: Average pH (A), ORP (mV) (B), and BOR (C) measurements for each steady state parameter
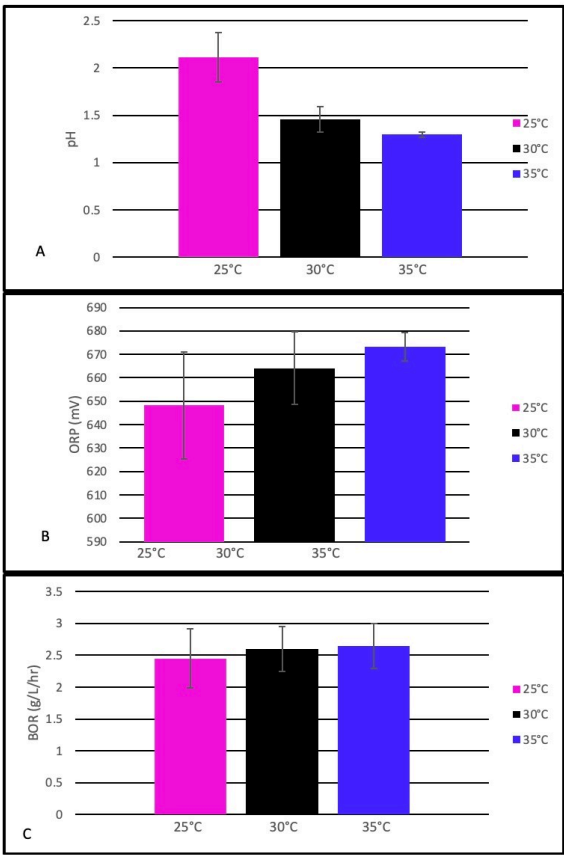
Figure 3: Average pH (A), ORP (mV) (B), and BOR (C) measurements for each steady state parameter

Figure 4: Biooxidation rate measurements for 40℃
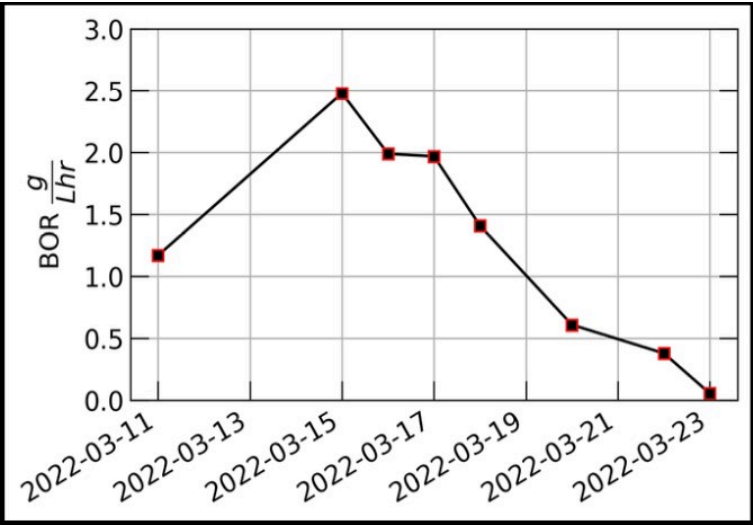
The overall mass balance (Figure 5a) shows high efficiency (above 90%) in all parameters. Likewise, element mass balance done with ICP-MS analysis showed a high percent return of all major elements in the solid and liquid sample (Figure 5b). Of the analyzed elements, iron had the lowest return and calcium had the highest overall steady state parameters.

Figure 5: Overall mass balance % efficiency (labeled as success) by weight (A) and element (B) showed high percent success
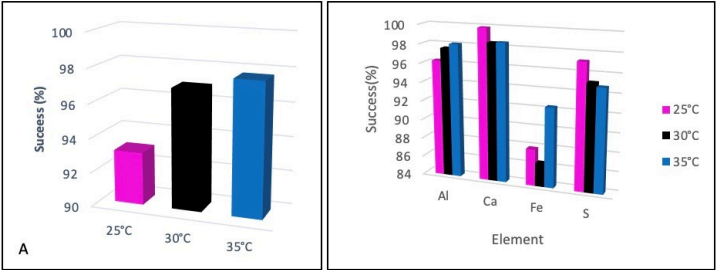
Figure 5: Overall mass balance % efficiency (labeled as success) by weight (A) and element (B) showed high percent success

Figure 6 shows SEM/EDX analysis of 25℃ and 35℃. These are the lowest and highest temperature parameters that achieved a steady state. 25℃ (Figure 5a) is shown at a magnification of 250x. Elemental analysis showed that there is a significant concentration of both iron and calcium on its surface. 35℃ (Figure 5b) is at a magnification of 500x. When compared to the elemental concentration of iron in 25℃, 35℃ has much less iron on its surface. The concentration of calcium is approximately the same as 25℃. XRD analysis confirmed the high concentrations of calcium, pyrite, and silica in all analyzed samples.

Figure 6: SEM images from 25℃ using magnification 250x (a) and 35℃ using magnification 500x (b)
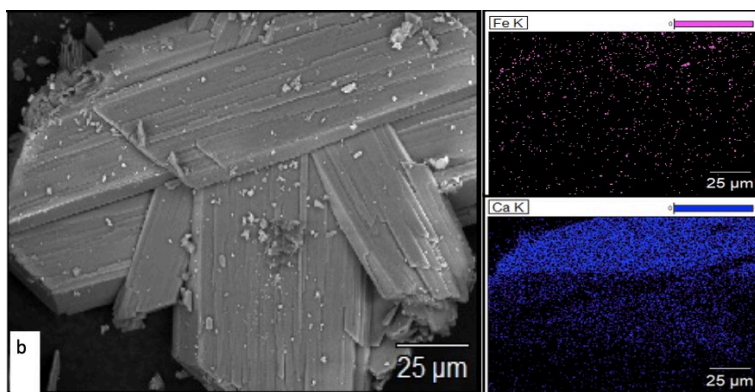
At the conclusion of this study, a bacterial assay was performed on the content of the bioreactor. The results, shown in Figure 7, indicate an overwhelming majority of Leptospirillium ferriphilum (89.58%), with smaller concentrations of Sulfobacillus benefaciens (5.35%) and Acidithiobacillus ferridurans (4.43%).

Figure 7: Bacterial assay of bioreactor culture showing bacterial populations in percent

Figure 7: Bacterial assay of bioreactor culture showing bacterial populations in percent

DISCUSSION

A low pH indicates more acid production in the bioreactor. This acid production aids in the biooxidation of the pyrite resulting in a positive feedback loop of acid production and pyrite oxidation. This is supported by the data shown in Figure 3 where a lower pH leads to more active (higher ORP) and faster-consuming (higher BOR) bacteria. By these standards, the parameter at 35℃ showed the best overall performance. There is a larger difference between the gathered [25℃ – 30℃] data than between [30℃ – 35℃]. This indicates that the minimum running temperature for the bioreactor is 30℃, while the optimal running temperature is 35℃. When the temperature was increased to 40℃, the biooxidation rate dropped dramatically (Figure 4). The initial increase may be attributed to machinery difficulties that were resolved prior to the start of the 40℃ parameter. These lingering effects may have contributed to this initial rise, as the bacteria experienced an increase in productivity after several days of no motor agitation. The initial increase was followed by a precipitous

drop in BOR over the next week. A zero BOR indicates bacterial death. The bioreactor should not run above 35℃ as it leads to inefficient pyrite consumption and eventual death of the bacteria.

The mass balances in Figure 5 show a high yield in both elementally and volumetrically. These results show that the experimental set-up used for all the parameters performed adequately for all recorded parameters.

Figure 6 shows SEM pictures of pyrite crystals from the low and high-temperature parameters (TP 25℃ and TP 35℃, respectively). A full EDX analysis was performed, but the most important element in this study was iron since the bacteria consume iron to survive. Conversely, the bacteria do not interact with calcium making it a good comparison element. Visually, the pyrite crystals from 35℃ look smoother than those from 25℃. This observation was confirmed with EDX analysis which shows that 35℃ has less iron on its surface than TP 25℃. The bacteria were more adept at oxidizing the pyrite at high temperatures as shown in the average BOR measurements in Figure 3. This resulted in the removal of iron from the surface.

At the inception of this study, a culture containing pure, Acidithiobacillus ferrooxidans, were housed in the bioreactor. However, Figure 7 shows that over time the bacterial species evolved to several species with the dominant one being Leptospirillium ferriphilum. During the two-year course of the bioreactor's performance before this study was conducted, there was no noticeable difference in its performance when held at steady state. Future studies should evaluate the difference and efficiencies between several biooxidizing bacteria to determine whether this evolution is triggered by any one component of the bioreactor's operating.

CONCLUSIONS

Alternative methods for REE extraction need to be explored due to the growing demand and environmental repercussions of current methods. Temperature is an important factor when extracting REE elements using biooxidation. Using a 3L bioreactor with 6% pyrite and a bacterial catalyst, the temperature was varied between 25℃ and 40℃ in increments of 5℃. An analysis of the pH, ORP, and BOR data showed that 35℃ is the optimal running temperature and results in the highest production of iron and acid from pyrite. 40℃ resulted in bacterial death and 25℃ produced suboptimal bioreactor performance. This conclusion was supported by SEM and XRD analysis of the pyrite crystals. Future work will include the analysis of rotation speed, bacterial culture, and solid percent on the performance of the bacteria.

REFERENCES

[1] N. Hidayah and S. Abidin, "The evolution of mineral processing in extraction of rare earth elements using solid-liquid extraction over liquid-liquid extraction: A review", Minerals Engineering, 112 103-113 (2017).

[2] S. Massari and M. Ruberti, "Ra [13] [13]re earth elements as critical raw materials: Focus on international markets and future strategies", Resources Policy, 38 [1] 36-43 (2013).

[3] L. Ferreira, T. Müller, M. Cargnin, C. de Oliveira and M. Peterson, "Valorization of waste from coal mining pyrite beneficiation", Journal of Environmental Chemical Engineering, 9 [4] 105759 (2021).

[4] Cockell, C.S., Santomartino, R., Finster, K. et al. Space station biomining experiment demonstrates rare earth element extraction in microgravity and Mars gravity. Nat Commun 11, 5523 (2020).

[5] A. Mazuelos, N. Iglesias and F. Carranza, "Inhibition of

bioleaching processes by organics from solvent extraction", Process Biochemistry, 35 [5] 425-431 (1999).

[6] R. Quatrini and D. Johnson, "Acidithiobacillus ferrooxidans", Trends in Microbiology, 27 [3] 282-283 (2019).

[7] J. Valdés, I. Pedroso, R. Quatrini, R. Dodson, H. Tettelin, R. Blake, J. Eisen and D. Holmes, "Acidithiobacillus ferrooxidans metabolism: from genome sequence to industrial applications", BMC Genomics, 9 [1] (2008).

[8] S. MOUSAVI, S. YAGHMAEI, F. SALIMI and A. JAFARI, "Influence of process variables on biooxidation of ferrous sulfate by an indigenous Acidithiobacillus ferrooxidans. Part I: Flask experiments", Fuel, 85 [17-18] 2555-2560 (2006).

[9] S. Mousavi, S. Yaghmaei and A. Jafari, "Influence of process variables on biooxidation of ferrous sulfate by an indigenous Acidithiobacillus ferrooxidans. Part II: Bioreactor experiments", Fuel, 86 [7-8] 993-999 (2007).

[10] S. Mohapatra, S. Bohidar, N. Pradhan, R. Kar and L. Sukla, "Microbial extraction of nickel from Sukinda chromite overburden by Acidithiobacillus ferrooxidans and Aspergillus strains", Hydrometallurgy, 85 [1] 1-8 (2007).

[11] S. Malhotra, A. Tankhiwale, A. Rajvaidya and R. Pandey, "Optimal conditions for biooxidation of ferrous ions to ferric ions using Thiobacillus ferrooxidans", Bioresource Technology, 85 [3] 225-234 (2002).

[12] C. Montes-Rosua, N. Iglesias-Gonzalez, A. Mazuelos, R. Romero and F. Carranza, "The effect of temperature on the bio-oxidation of mining effluents containing tetrathionate", Hydrometallurgy, 178 37-42 (2018).k

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26. **Research Reflection by Kitsel Lusted**

Kitsel Lusted

Faculty Mentor: Michael Free (Materials Science and Engineering, University of Utah)

The research I conducted with Dr. Free was the best possible experience I could have as an undergraduate. During my time in the lab, I acquired invaluable exposure to running and managing a bioreactor lab, including the ordering of supplies and proper disposal of chemicals. I learned hard lessons about research and how to deal with obstacles when research gets tough. Dr. Free has been instrumental to my success. Through our weekly group meetings, all of us in the lab were able to get his input on the progress of the study. He is an incredible P.I., who has given me so many professional and personal opportunities. Through Dr. Free's lab, I was able to present research to legislators at the Utah Capitol. I will also be attending my first conference as a presenter with this research

in August.

I plan to go to graduate school where I will study geophysics. I am particularly interested in glacial hydrology and the environmental impacts of climate change. My undergraduate research helped solidify that I wanted to pursue a research degree. None of it would have been possible without Dr. Free along with the rest of the people in our lab and I want to thank them from the bottom of my heart for a wonderful formative research experience.

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27. **Importance Sampling Techniques for the Analysis of Genetic Circuits**

Payton Thomas

Faculty Mentor: Chris Myers (Electrical & Computer Engineering, University of Utah)

Abstract

Genetic circuits have been identified as a promising technology that could revolutionize several areas, including biofuels, biomanufacturing, and medicine. Despite the tremendous potential of this technology, there are significant challenges associated with its development and implementation. One of the major challenges with genetic circuits is the stochastic nature of their behavior. Unlike traditional engineering systems, genetic circuits are inherently

probabilistic, which makes it more challenging to design and verify their performance. In particular, genetic circuits can exhibit rare failure states known as glitches, which are difficult to predict and prevent. To address this challenge, multiple stochastic simulation algorithms have been developed that use importance sampling as a variance reduction technique to estimate the probability of glitching behavior. However, these methods are often unreliable and impractical for use in design workflows. As a result, a mathematical analysis of these methods is necessary to identify the reasons why they fail to rapidly estimate low probabilities. To meet this need, we performed a comprehensive mathematical analysis of these methods to determine why they are ineffective in estimating low probabilities and to showcase their strengths and shortcomings. To achieve this end, we developed several case studies highlighting the challenges associated with these methods and their potential applications in genetic circuit design. This analysis provides crucial insights into the development of more effective algorithms for estimating the probability of rare glitching behaviors in genetic circuits. The results of this work will guide the future development of more applicable algorithms that can be used in design workflows to improve the reliability and predictability of genetic circuits. By overcoming the challenges associated with the stochastic nature of genetic circuits, these algorithms will enable the widespread adoption of genetic circuit technology in various fields, including biofuels, biomanufacturing, and medicine.

Introduction

Genetic circuits are synthetic DNA constructs which implement engineered computational or logical functions in living cells. Genetic circuits promise to revolutionize biofuels, biomanufacturing, and medicine by improving biochemical

production [1]. Inspired by traditional engineering disciplines, synthetic biologists utilize designbuild- test-learn (DBTL) cycles to yield useful genetic circuits [2]. The design phase of the DBTL cycle may include modeling. Modeling steps are beneficial to the design process because they allow for design verification before any circuit is actually built, reducing the necessary number of orbits around the DBTL cycle. To be useful, models of genetic circuits must be able to accurately predict whether or not a given design will fail. Predicting genetic circuit failures can be difficult, however, because chemical reaction networks, including genetic circuits, are inherently stochastic due to the random movements of molecules [3]. This stochasticity is responsible for rare failure states in genetic circuits known as glitches, which occur with some probability and may be devastating to a biological system [4, 5].

The probability of a glitch may be estimated by simulating many system trajectories with the stochastic simulation algorithm (SSA) [6, 7]. For rare events the number of trajectories necessary to precisely estimate the probability of failure may be computationally prohibitive. The problem of estimating the probability of rare events has prompted the creation of several algorithms which utilize the statistical technique of importance sampling (IS) [8] to more rapidly estimate low probabilities. These techniques include theWeighted SSA (wSSA) [9], State-dependent Weighted SSA (swSSA) [10], and Guided wSSA [11, 12].

These algorithms have been applied applied to a few toy models [9–12], but none have been shown to be suitable for use in genetic circuit design verification. To be used in genetic design verification, an IS-based stochastic simulation algorithm must be able to accurately and precisely estimate the

probability of rare failure states in genetic circuits, must be straightforward to apply, and must be sufficiently stable and robust. The existing IS-based algorithms must therefore be tested for these qualities.

To this end, we implemented each algorithm and benchmarked its performance on a number of case studies varying from toy models to genetic circuits. This revealed fundamental mathematical challenges which the algorithms fail to overcome. Equipped with these results, we formed recommendations for more robust IS schemes. Ultimately, this work may lead to effectual simulation methods which may be used in conjunction with stochastic model checking to accelerate the design of genetic circuits. Accelerated design protocols will prove crucial as genetic circuits are applied to diverse biochemical production tasks.

Background

Genetic Circuits Genetic circuits are synthetic collections of biological components, inspired by natural gene regulatory networks, which implement computational functions in living cells. Early examples of genetic circuits include the genetic toggle switch [13] and repressilator [14]. Over the past two decades, genetic circuits have evolved beyond simple operations to solve problems in diverse fields [1].

Digital logic circuits are often used as models of genetic circuits. The simplicity of this abstraction is appealing, but it neglects temporal dynamics and stochasticity. Nevertheless, digital logic models have proven useful in the design of genetic circuits. Working within the abstraction of digital logic circuits, Nielsen et al. developed Cello, a genetic design automation tool which produces circuit designs from a user-specified logic table. To validate Cello's performance, Nielsen et al. produced 60 genetic circuits using the tool [15]. One of these circuits,

0x8E, has been shown to exhibit glitching behaviors [4, 5] and is analyzed here.

2.1 Importance Sampling

Importance sampling is a Monte Carlo technique for rapidly estimating the properties of some distribution using samples from another distribution [8]. Importance sampling, in general, operates as follows. Suppose X is a random variable with sample space Ω which is distributed according to probability density function f(x). Further suppose that we want P{X ∈ E} where P{·} denotes the probability and E ⊂ Ω. We may write

$$P\{X \in E\} = Ef[I(x)],$$

where Ef [°] denotes the expected value under distribution f and I(x) is the indicator function

$$I(x) = \begin{cases} 1 & x \in E \\ 0 & x \notin E \end{cases}.$$

Let g(x) be a probability density function over the sample space Ω which is almost-everywhere nonzero. g is referred to as the importance density. Observe that

$$E_f[I(x)] \triangleq \int_\Omega I(x)f(x)dx = \int_\Omega I(x)\frac{f(x)}{g(x)}g(x)dx \triangleq E_g\left[I(x)\frac{f(x)}{g(x)}\right].$$

Let w(x) = f(x) / g(x) be the weight function, so we may write

$$P\{X \in E\} = E_g[I(x)w(x)].$$

By the law of large numbers,

$$E_g[I(x)w(x)] = \lim_{N \to \infty} \frac{1}{N} \sum_{n=1}^{N} I(x_n)w(x_n),$$

where each xn is a sample from g. This yields the unbiased estimator

$$\hat{p} = \frac{1}{N} \sum_{n=1}^{N} I(x_n)w(x_n) \quad (\forall n, x_n \sim g),$$

which, given a good choice in g, may have lower variance than the naive sample estimator.

2.2 The SSA The SSA [6, 7] is an algorithm that produces exact trajectories of chemical reaction networks. The SSA is developed as follows. Suppose we have a chemical reaction network with n chemical species {S1, . . . , Sn} and m reactions {R1, . . . ,Rm} with corresponding reaction rate constants {a1, . . . , am} (referred to as propensity functions within the context of stochastic chemical kinetics). Let Xi(t) be a random variable representing the number of molecules of Si at time t. We write X(t) = (X1(t), . . . ,Xn(t)). Additionally, each reaction channel Rμ has an associated state-change vector vμ = (v1μ, . . . , vnμ) where viμ is the change in Xi following a turn of reaction Rμ. The state-change vectors are arranged to form the stoichiometric matrix

$$S = \begin{pmatrix} | & & | \\ \mathbf{v}_1 & \cdots & \mathbf{v}_n \\ | & & | \end{pmatrix}.$$

Let $p(\tau, \mu|x, t)d\tau$ denote the probability the the next reaction which occurs in a network is $R\mu$ and that it will occur on the interval $[t + \tau, t + \tau + d\tau]$, given that $X(t) = x$. We may write

$$p(\tau, \mu|\mathbf{x}, t)d\tau = P_0(\tau|\mathbf{x}, t) \times a_\mu(\mathbf{x})d\tau,$$

where $P0(\tau |x, t)$ denotes the probability that no reaction occurs on the interval $[t, t + \tau)$. Observe that $P0$ must satisfy

$$P_0(\tau + d\tau|\mathbf{x}, t) = P_0(\tau|\mathbf{x}, t) \times \left[1 - \sum_{i=1}^{m} a_i(\mathbf{x})d\tau \right]$$

$$\implies P_0(\tau + d\tau|\mathbf{x}, t) - P_0(\tau|\mathbf{x}, t) = -P_0(\tau|\mathbf{x}, t) \sum_{i=1}^{m} a_i(\mathbf{x})d\tau$$

$$\implies \frac{dP_0(\tau|\mathbf{x}, t)}{d\tau} = -a_0(\mathbf{x})P_0(\tau|\mathbf{x}, t),$$

where $a_0(\mathbf{x}) = \sum_{i=1}^{m} a_i(\mathbf{x})$. We impose the initial condition $P_0(0|\mathbf{x}, t) = 1$ to derive

$$P_0(\tau, \mathbf{x}, t) = e^{-a_0(\mathbf{x})\tau}.$$

We may therefore write

$$p(\tau, \mu|\mathbf{x}, t) = e^{-a_0(\mathbf{x})\tau} \times a_\mu(\mathbf{x}) = a_0(\mathbf{x})e^{-a_0(\mathbf{x})\tau} \times \frac{a_\mu(\mathbf{x})}{a_0(\mathbf{x})}.$$

This reveals that τ and μ are independently and randomly distributed, and that τ is exponentially distributed with mean 1 a0(x) and μ is distributed such that each reaction Rμ has probability mass aμ(x)/a0(x). These observations, yield the SSA (Algorithm 1).

2.3 Weighted SSA Variants

2.3.1 The wSSA The wSSA

The wSSA [9] first applied IS to chemical reaction networks. The wSSA achieves this by assigning a biasing factor δμ to each reaction Rμ. These biasing factors are applied multiplicatively to the propensity functions aμ to form predilection functions

$$b\mu(x) = \delta\mu a\mu(x).$$

Reactions are then selected according to these predilection functions, rather than the true propensities. In the wSSA, predilection functions are not used to determine interevent time τ. Each reaction Rμ therefore has weight function

$$w(\mu, \mathbf{x}) = \frac{a_j(\mathbf{x})/a_0(\mathbf{x})}{b_j(\mathbf{x})/b_0(\mathbf{x})} = \frac{a_j(\mathbf{x})b_0(\mathbf{x})}{a_0(\mathbf{x})b_j(\mathbf{x})},$$

where b0(x) = Pm i=1 bi(x) If a given system trajectory has reaction sequence (Rμ(1) , . . . ,Rμ(k)), then the total weight function corresponding to the trajectory is

$$w = \prod_{i=1}^{k} \frac{a_j(\mathbf{x})b_0(\mathbf{x})}{a_0(\mathbf{x})b_j(\mathbf{x})}.$$

These modifications to the SSA yield the wSSA (Algorithm 2).

2.3.2 The swSSA

The wSSA applies constant multiplicative biasing to each propensity function to yield predilection functions [10]. This is limited because it does not account for whether the current state x causes a given propensity function aμ(x) be large or small prior to any biasing. To improve the robustness of the wSSA, the swSSA utilizes a statedependent biasing factor as follows. Let ρμ(x) = aμ(x)/a0(x) be the relative propensity. Prior to simulation, reactions to be biased upward,downward, and not at all are sorted into sets GE, GD and GN, respectively. For each reaction Rμ ∈ GE ∪ GD, a threshold ρ0 μ and maximal biasing γmax μ are selected. For Rμ ∈ GE, the dynamic multiplicative biasing factor

$$\delta_\mu(\rho_\mu) = \begin{cases} 1 & \rho_\mu(\mathbf{x}) \geq \rho_\mu^0 \\ f_\mu(\rho_\mu(\mathbf{x})) & \rho_\mu(\mathbf{x}) < \rho_\mu^0 \end{cases}$$

where fμ(ρμ(x)) is parabolic function with

1. $f_\mu(0) = \gamma_\mu^{max}$

2. $f_\mu(\rho_\mu^0) = 1$

For Rμ ∈ GD, the dynamic multiplicative biasing factor

$$\delta_\mu(\rho_\mu) = \begin{cases} 1 & \rho_\mu(\mathbf{x}) < \rho_\mu^0 \\ g_\mu(\rho_\mu(\mathbf{x})) & \rho_\mu(\mathbf{x}) \geq \rho_\mu^0 \end{cases}$$

where $g_\mu(\rho_\mu(x))$ is a parabolic function with

1. $g_\mu(1) = 1/\gamma_\mu^{max}$

2. $g_\mu(\rho_\mu^0) = 1$

(Figure 1). These modifications to the wSSA yield the swSSA (Algorithm 3).

2.3.3 The Guided wSSA

Both the wSSA and swSSA rely on the user to select biasing parameters based on some a priori knowledge of the network being simulated. The Guided wSSA [11,12] automatically biases reactions at each point in simulation to circumvent this reliance. This biasing is designed to, on average, bring the chemical reaction network to the state of interest immediately before the maximum simulation time. This is achieved by assuming that the number of times each reaction will occur before the maximum simulation time is Poisson distributed. A multivariate normal approximation to the Poisson distribution is used to calculate the expected number of times each reaction will occur before maximum simulation time given that the state of interest is reached at maximum simulation time. The details of this formulation require that the state of interest be a condition on a linear combination of chemical species, that is, there exists an F such that the state of interest is achieved when

FT x reaches some value. These modifications to the wSSA yield
the Guided wSSA (Algorithm 4). H denotes diag(b(x)).

Figure 1: Parabolic f and g function utilized by the swSSA.
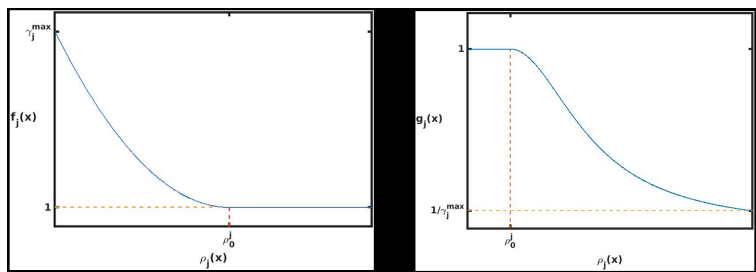
The published version of the Guided wSSA is unstable,
however, because it routinely selects negative biasing
parameters, which cannot be used to select a reaction.
Inspection of the original Guided wSSA implemented, provided
by the authors of the Guided wSSA paper [11], reveals that
three unmotivated methods were used to remove negative
predilections. In all three methods, the effective biasing factors

$$\delta_\mu = \frac{b_\mu(\mathbf{x})}{a_\mu(\mathbf{x})}$$

were calculated. In one case, the 0.01 plus absolute value of
the most negative biasing factor was added to all biasing factors
so that all biasing factors became positive. In another case,
all biasing factors were divided by the most negative biasing
factor, which only eliminates negatives if all biasing factors
share the same sign. In yet another case, all negative biasing

factors were replaced with one. After the biasing factors were modified, the predilections were recalculated as usual:

$$b_\mu(\mathbf{x}) = \delta_\mu a(\mathbf{x}).$$

The final method is used in analyses included in this paper.

3 Methods

3.1 Model Biochemical Systems

3.1.1 Enzymatic Futile Cycle

The enzymatic futile cycle is a simple biochemical motif present in a variety of biology pathways, including GTPase cycles, MAP Kinase cascades, and glucose mobilization [16]. The enzymatic futile cycle is therefore an archetypal naturally occurring biochemical system with few reactions, and is given as follows:

$$R_1 : S_1 + S_2 \xrightarrow{k_1} S_3,$$
$$R_2 : S_3 \xrightarrow{k_2} S_1 + S_2,$$
$$R_3 : S_3 \xrightarrow{k_3} S_1 + S_5,$$
$$R_4 : S_4 + S_5 \xrightarrow{k_4} S_6,$$
$$R_5 : S_6 \xrightarrow{k_5} S_4 + S_5,$$
$$R_6 : S_6 \xrightarrow{k_6} S_4 + S_2.$$
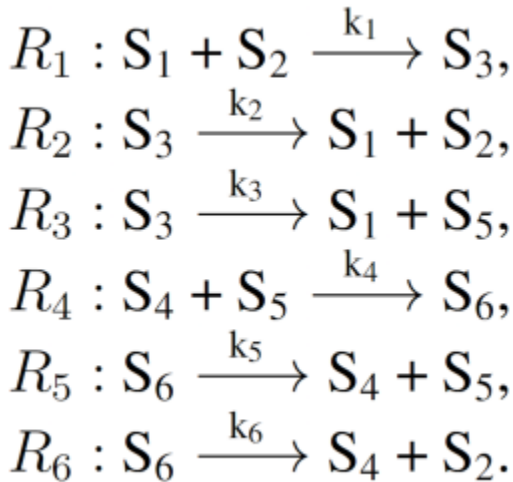
where

$$k_1 = k_2 = k_4 = k_5 = 1, \quad k_3 = k_6 = 0.1.$$

The rare event of interest is S5 falling to 25 molecules or fewer before 100 time units have passed, subject to the following initial conditions:

$$S_1(0) = S_4(0) = 1, \quad S_2(0) = S_5(0) = 50,$$

$$S_3(0) = S_6(0) = 0.$$

This event is rare because the symmetry of the initial molecule counts and reaction rate constants keeps the system near its initial condition with high probability. The probability of this event has been previously calculated to be 1.71×10^{-7} [9]. We consider this value to be exact.

To apply the wSSA to this model, each reaction must be assigned a multiplicative biasing parameter. We used use a single biasing parameter $\delta \in (0, 1)$ to simplify parameter optimization to one dimension. δ was be applied to reactions to be biased downward and δ^{-1} was applied to reactions to be biased upward. This simplification was made for all models. In the enzymatic futile cycle, S2 production may be biased upward and S5 production may be biased downward to increase the probability of S5 falling to 25. This gives the following wSSA weighting scheme:

$$b_1(\mathbf{x}) = a_1(\mathbf{x}), \quad b_2(\mathbf{x}) = a_2(\mathbf{x}), \quad b_3(\mathbf{x}) = \delta a_3(\mathbf{x})$$

$$b_4(\mathbf{x}) = a_4(\mathbf{x}), \quad b_5(\mathbf{x}) = a_5(\mathbf{x}), \quad b_6(\mathbf{x}) = \frac{1}{\delta} a_6(\mathbf{x}).$$

The swSSA also requires biasing parameters to be specified. Unlike the wSSA, the swSSA biases reaction in a state-dependent manner, so the user must specify a relative propensity threshold beyond which biasing is applied and a maximal biasing value. For all models, we will use a fixed threshold of $\rho 0$ D = 0.15 for reactions that are being biased downward and $\rho 0$ E = 0.5 for reactions that are being biased upward. The maximal biasing value γmax can then be varied to again simplify parameter optimization to one dimension. This gives the following biasing scheme for the futile cycle:

$$b_1(\mathbf{x}) = a_1(\mathbf{x}), \quad b_2(\mathbf{x}) = a_2(\mathbf{x}), \quad b_3(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_3(\mathbf{x})$$

$$b_4(\mathbf{x}) = a_4(\mathbf{x}), \quad b_5(\mathbf{x}) = a_5(\mathbf{x}), \quad b_6(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_6(\mathbf{x})$$

3.1.2 Modified Yeast Polarization

The modified yeast polarization model represents the pheromone induced G-protein cycle in Saccharomyces cerevisia [17]. This model is a more complicated naturally occurring biochemical reaction network than the futile cycle, and is given as follows:

$$R_1 : \emptyset \xrightarrow{\text{k}_{Rs}} R,$$

$$R_2 : R \xrightarrow{k_{Rd}} \emptyset,$$

$$R_3 : L + R \xrightarrow{\text{k}_{RLL}} RL + L,$$

$$R_4 : RL \xrightarrow{\text{k}_R} R,$$

$$R_5 : RL + G \xrightarrow{k_{Ga}} G_a + G_{bg},$$

$$R_6 : G_a \xrightarrow{k_{Gd}} G_d,$$

$$R_7 : G_d + G_{bg} \xrightarrow{\text{k}_G} G,$$

$$R_8 : \emptyset \xrightarrow{k_{RL}} RL$$

where

$$k_{R_s} = 0.0038, \quad k_{R_d} = 4.00 \times 10^{-4}, \quad k_{RLL} = 0.042, \quad k_R = 0.0100$$
$$k_{G_a} = 0.011, \quad k_{G_d} = 0.100, \quad k_G = 1.05 \times 10^3, \quad k_{RL} = 3.21.$$

The initial state of the model is set as follows:

$$b_1(\mathbf{x}) = \frac{1}{\delta} a_1(\mathbf{x}), \quad b_2(\mathbf{x}) = \delta a_2(\mathbf{x}), \quad b_3(\mathbf{x}) = \frac{1}{\delta} a_3(\mathbf{x})$$

$$b_4(\mathbf{x}) = \delta a_4(\mathbf{x}), \quad b_5(\mathbf{x}) = \frac{1}{\delta} a_5(\mathbf{x}), \quad b_6(\mathbf{x}) = \delta a_6(\mathbf{x})$$

$$b_7(\mathbf{x}) = \delta a_7(\mathbf{x}), \quad b_8(\mathbf{x}) = \frac{1}{\delta} a_8(\mathbf{x})$$

This scheme yields the following analogous swSSA scheme:

$$b_1(\mathbf{x}) = a_1(\mathbf{x}), \quad b_2(\mathbf{x}) = a_2(\mathbf{x}), \quad b_3(\mathbf{x}) = a_3(\mathbf{x})$$

$$b_4(\mathbf{x}) = a_4(\mathbf{x}), \quad b_5(\mathbf{x}) = a_5(\mathbf{x}), \quad b_6(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x}) a_6(\mathbf{x})$$

$$b_7(\mathbf{x}) = a_7(\mathbf{x}), \quad b_8(\mathbf{x}) = a_8(\mathbf{x})$$

3.1.3 Simplified Motility Regulation

The simplified motility regulation model represents the naturally occurring gene regulatory network which regulates flagella formation in Bacillus subtilis. This model serves as a a an archetypal gene regulatory network, and is given as follows:

$$R_1 : \text{codY} \xrightarrow{k_1} \text{codY} + \text{CodY},$$
$$R_2 : \text{CodY} \xrightarrow{k_2} \emptyset,$$
$$R_3 : \text{flache} \xrightarrow{k_3} \text{flache} + \text{SigD},$$
$$R_4 : \text{SigD} \xrightarrow{k_4} \emptyset,$$
$$R_5 : \text{SigD\_hag} \xrightarrow{k_5} \text{SigD} + \text{hag} + \text{Hag},$$
$$R_6 : \text{Hag} \xrightarrow{k_6} \emptyset,$$
$$R_7 : \text{SigD} + \text{hag} \xrightarrow{k_7} \text{SigD\_hag},$$
$$R_8 : \text{SigD\_hag} \xrightarrow{k_8} \text{SigD} + \text{hag},$$
$$R_9 : \text{CodY} + \text{flache} \xrightarrow{k_9} \text{CodY\_flache},$$
$$R_{10} : \text{CodY\_flache} \xrightarrow{k_{10}} \text{CodY} + \text{flache},$$
$$R_{11} : \text{CodY} + \text{hag} \xrightarrow{k_{11}} \text{CodY\_hag},$$
$$R_{12} : \text{CodY\_hag} \xrightarrow{k_{12}} \text{CodY} + \text{hag}$$
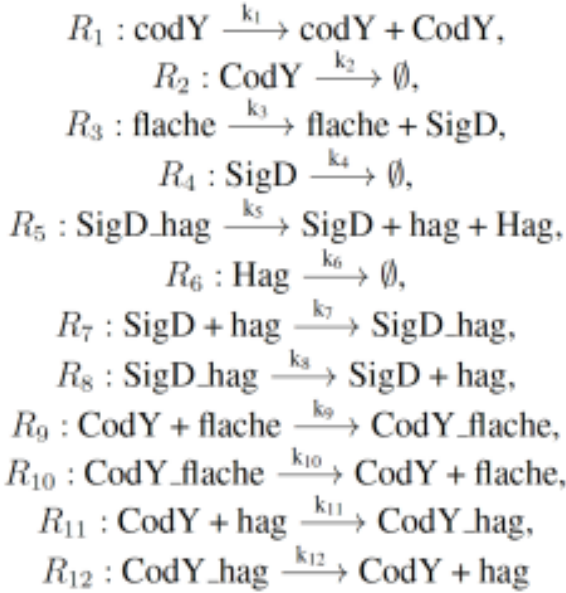
where

$$k_1 = k_8 = k_{10} = k_{12} = 0.1, \quad k_2 = k_4 = k_6 = 0.0002$$
$$k_3 = k_5 = 1, \quad k_7 = k_{11} = 0.01, \quad k_9 = 0.02.$$

The rare event of interest is CodY reaching 20 molecules before 10 time units have passed. Subject to the following initial conditions:

$$codY(0) = flache(0) = SigD\_hag(0) = hag(0) = CodY\_flache(0) = CodY\_hag(0) = 1,$$
$$CodY(0) = SigD(0) = Hag(0) = 10.$$

The probability of this event has been previously calculated to be 2.161 × 10−7 [11]. We will consider this value to be exact. In the simplified motility regulation model, codY transcription/translation, Cod flache dissociation, and CodY hag dissociation may be increased and CodY degradation, CodY flache association, and CodY hag association may be decreased to increase the probability that CodY rises to 20. This gives the following wSSA weighting scheme:

$$b_1(\mathbf{x}) = \frac{1}{\delta}a_1(\mathbf{x}), \quad b_2(\mathbf{x}) = \delta a_2(\mathbf{x}), \quad b_3(\mathbf{x}) = a_3(\mathbf{x})$$
$$b_4(\mathbf{x}) = a_4(\mathbf{x}), \quad b_5(\mathbf{x}) = a_5(\mathbf{x}), \quad b_6(\mathbf{x}) = a_6(\mathbf{x})$$
$$b_7(\mathbf{x}) = a_7(\mathbf{x}), \quad b_8(\mathbf{x}) = a_8(\mathbf{x}), \quad b_9(\mathbf{x}) = \delta a_9(\mathbf{x})$$
$$b_{10}(\mathbf{x}) = \frac{1}{\delta}a_{10}(\mathbf{x}), \quad b_{11} = \delta a_{11}(\mathbf{x}), \quad b_{12}(\mathbf{x}) = \frac{1}{\delta}a_{12}(\mathbf{x})$$

Again, we fix ρ0 D = 0.15 for all reactions selected to be biased downward and ρ0 E = 0.5 for all reactions selected to be biased upward. this gives the following swSSA weighting scheme:

$$b_1(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_1(\mathbf{x}), \quad b_2(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_2(\mathbf{x}), \quad b_3(\mathbf{x}) = a_3(\mathbf{x})$$

$$b_4(\mathbf{x}) = a_4(\mathbf{x}), \quad b_5(\mathbf{x}) = a_5(\mathbf{x}), \quad b_6(\mathbf{x}) = a_6(\mathbf{x})$$

$$b_7(\mathbf{x}) = a_7(\mathbf{x}), \quad b_8(\mathbf{x}) = a_8(\mathbf{x}), \quad b_9(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_9(\mathbf{x})$$

$$b_{10}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{10}(\mathbf{x}), \quad b_{11} = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_{11}(\mathbf{x}), \quad b_{12}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{12}(\mathbf{x})$$

3.1.4 Genetic Circuit 0x8E

Genetic circuit 0x8E is one of 60 genetic digital logic circuits designed using the genetic design automation tool Cello [15]. Circuit 0x8E has three input molecules Arabinose (Ara, ChEBI = 17535), Isopropyl-beta-Dthiogalactopyranoside (IPTG, ChEBI=61448) and Acetylcholine (aTc, ChEBI=15355), and one output, yellow fluorescent protein (YFP). This model serves as a archetypal genetic circuit. The input molecules have to be present for a prolonged time so that the input states can propagate through the different levels of logic. The circuit has three input molecules and therefore eight input combinations: (IPTG, aTc, Ara) ∈ {(0,0,0), (0,0,1), (0,1,0), (0,1,1), (1,0,0), (1,0,1), (1,1,0), (1,1,1)}, where one indicates the presence of a molecule and zero represents the absence of a molecule. Of these eight different input combinations, four result in the production of YFP and four do not. The circuit produces no YFP at steady state when (IPTG, aTc, Ara) = (1, 0, 0) or when (IPTG, aTc, Ara) = (1, 1, 1). However, if the circuit takes (IPTG, aTc, Ara) = (1, 0, 0) as an input and is then transitioned to (IPTG, aTc, Ara) = (1, 1, 1), YFP is transiently expressed before the circuit once again reacher steady state [4,5,15]. This unpredicted and unwanted behavior is known as a glitch. The genetic circuit 0x8E model consists of 15 reactions including 79 reaction rates. The rare event of interest is the quantification of that glitching behavior by determining the probability of YFP exceeding 70 molecules before 1000 time units pass. The probability of this event was estimated to be 6.29×10^{-4} with 10^6 runs of the SSA. We will consider this value to be exact. YFP production may

be increased and YFP degradation may be decreased to increase the probability that YFP rises to 70. This gives the following wSSA weighting scheme:

$$b_{13}(\mathbf{x}) = \frac{1}{\delta}a_{13}(\mathbf{x}), \quad b_{14}(\mathbf{x}) = \frac{1}{\delta}a_{14}(\mathbf{x}), \quad b_{15}(\mathbf{x}) = \delta a_{15}(\mathbf{x})$$

with all other reactions unbiased. Again fixing ρ0 D = 0.15 and ρ0 E = 0.5 gives the following swSSA weighting scheme:

$$b_{13}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{13}(\mathbf{x}), \quad b_{14}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{14}(\mathbf{x}), \quad b_{15}(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_{15}(\mathbf{x})$$

3.1.5 Genetic Circuit 0x8E LHF

Genetic circuit 0x8E LHF is a Logic Hazard Free redesign of genetic circuit 0x8E [4]. The redesign utilized hazard-preserving optimizations to greatly reduce the glitching probability without changing the input molecules or stead state outputs of the original circuit 0x8E.

The model consists of 16 reactions including 94 reaction rates. The rare event of interest is the quantification of the glitching behavior by determining the probability of YFP exceeding 160 molecules before 1000 time units pass. The probability of this event was estimated to be 7.33 × 10−4 with 107 runs of the SSA. We will consider this value to be exact.

YFP production may be increased and YFP degradation may be decreased to increase the probability that YFP rises to 160. This gives the following wSSA weighting scheme:

$$b_{14}(\mathbf{x}) = \frac{1}{\delta}a_{14}(\mathbf{x}), \quad b_{15}(\mathbf{x}) = \frac{1}{\delta}a_{15}(\mathbf{x}), \quad b_{16}(\mathbf{x}) = \delta a_{16}(\mathbf{x})$$

with all other reactions unbiased. Fixing $\rho_D^0 = 0.15$ and $\rho_E^0 = 0.5$ gives the following swSSA weighting scheme:

$$b_{14}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{14}(\mathbf{x}), \quad b_{15}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{15}(\mathbf{x}), \quad b_{16}(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_{16}(\mathbf{x})$$

3.1.6 Genetic Circuit 0x8E TI

Genetic Circuit 0x8E TI is a redesign of genetic circuit 0x8E with Two added Inverters to add extra delay to a pathway of

the circuit to reduce its glitching behavior [4]. Like genetic circuit 0x8E LHF, genetic circuit 0x8E TI has reduced glitching probability compared to the original circuit 0x8E, but has the same inputs and output.

The genetic circuit 0x8E TI model consists of 19 reactions including 109 reaction rates. The rare event of interest is the quantification of the glitching behavior by determining the probability of YFP exceeding 100 molecules before 1000 time units pass. The probability of this event was estimated to be 8.74×10^{-4} with 10^7 runs of the SSA. We will consider this value to be exact.

YFP production may be increased and YFP degradation may be decreased to increase the probability that YFP rises to 100. This gives the following wSSA weighting scheme:

$$b_{17}(\mathbf{x}) = \frac{1}{\delta}a_{17}(\mathbf{x}), \quad b_{18}(\mathbf{x}) = \frac{1}{\delta}a_{18}(\mathbf{x}), \quad b_{19}(\mathbf{x}) = \delta a_{19}(\mathbf{x})$$

with all other reactions unbiased. Fixing $\rho_D^0 = 0.15$ and $\rho_E^0 = 0.5$ gives the following swSSA weighting scheme:

$$b_{17}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{17}(\mathbf{x}), \quad b_{18}(\mathbf{x}) = f(\rho_E^0, \gamma^{max}, \mathbf{x})a_{18}(\mathbf{x}), \quad b_{19}(\mathbf{x}) = g(\rho_D^0, \gamma^{max}, \mathbf{x})a_{19}(\mathbf{x})$$

3.2 Algorithmic Benchmarks
3.2.1 Biasing Parameter Optimization

For each model, we ran the wSSA with 30 values of δ from 0.05 to 1.5. Because the true probability of each rare event of interest is known, we may draw conclusions about which value(s) of δ performed most optimally for each model. The number of simulation runs was selected such that 'good' and 'bad' biasing parameters were easily discernible. We may also use these results to make claims about the robustness of each algorithm to biasing parameter variation. Similarly, for each model, we ran the swSSA an appropriate number of times with 33 values of γmax. The domain of these values was varied from model to model to attain optimal performance. As with the wSSA, we may draw conclusions about which value(s) of

γmax performed most optimally and about the robustness of the swSSA.

3.2.2 Convergence Comparison

For each model, we performed 104 simulation runs with each algorithm. We selected this number of runs because it was sufficient for some but not all of the algorithms considered here to estimate the probability of the event of interest well in all models. After each simulation run, we calculated the estimated probability of the rare event of interest so that the rate of convergence of each algorithm could be visually compared. For these simulations, the wSSA and swSSA were given biasing parameters determined to be optimal.

3.2.3 Run Weight Variance Comparison

To compare the accuracy and precision of each method, we performed 103 simulation runs on each model. We then calculated the estimated probability of each model's rare event of interest as well as the variance in the IS weight of simulation runs. Run weight variance has traditionally been used as a measure for biasing scheme optimality [9–11]. 3.2.4 Runtime Efficiency Comparison For each model, we performed 103 simulation runs for each algorithm and recorded the total run time (all simulation were performed on a machine with an Intel i7 4-core 2.11GHz processor and 8 GB of RAM, running Windows 10 Pro (v21H1)). This number of runs was selected because it yielded a run time on the order of seconds for most algorithms on most models. This revealed the run time efficiency of each algorithm as well as how that efficiency scales with model size.

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4 Results

4.1 Enzymatic Futile Cycle

We first applied each importance sampling (IS) algorithm to the enzymatic futile cycle model (Section 3.1.1) to determine the probability of S5 falling to 25 molecules or fewer before 100 time units have passed. The enzymatic futile cycle is a typical biochemical motif, so these results are reflective of each algorithm's ability to model rare events in simple biochemical systems. The probability of the event of interest in this model is $p = 1.71 \times 10{-7}$.
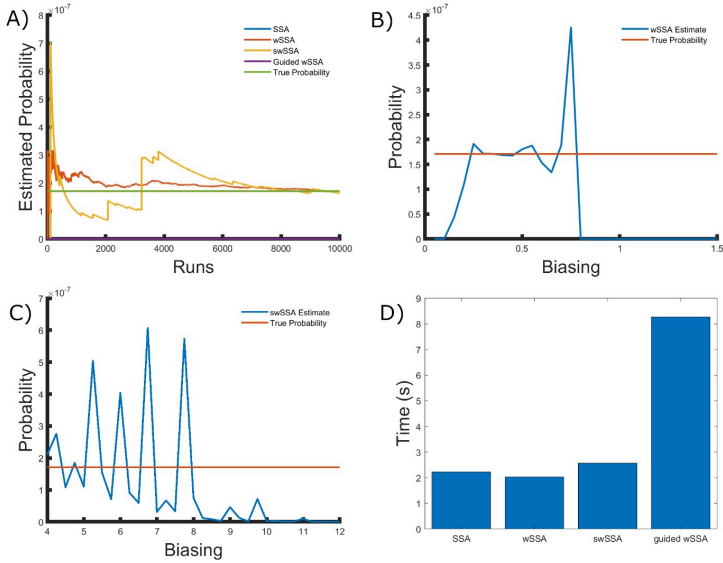
Figure 2: Simulation results for the enzymatic futile cycle model (true probability 1.71×10^{-7}). (A) Convergence of Monte Carlo estimated probability for the SSA, wSSA, swSSA, and Guided wSSA over 10^4 runs ($\delta = 0.5$, $\gamma_{max} = 2.5$). (B) Probability estimated by the wSSA at 10^4 runs as a function of δ. (C) Probability estimated by the swSSA at 10^4 runs as a function of γ_{max}. (D) Time for the SSA, wSSA, swSSA, and Guided wSSA to complete 10^3 runs.

We found that the wSSA and swSSA estimated p much faster than the SSA after 10^4 runs, but were sensitive to biasing parameters, while the Guided wSSA performed worse than the SSA. Biasing parameter optimization (Section 3.2.1) revealed that the wSSA and swSSA performed near optimally with biasing parameters $\delta = 0.5$ and $\gamma_{max} = 2.5$, respectively. However, both algorithms exhibited high relative error and poor performance as their biasing parameters varied from these values (Figure 2B and C). There is no existing technique for deriving these optimal biasing parameters without first

knowing p. Convergence comparison (Section 3.2.2 revealed that, with these near-optimal biasing parameters, the wSSA and swSSA estimated p well within 104 runs, while the SSA and Guided wSSA failed to reach the state of interest even once (Figure 2A). The enhanced run efficiency of the wSSA and swSSA relative to the SSA does indicate that the user may estimate p more rapidly with these methods because the wSSA and swSSA performed with similar runtime efficiency (Section 3.2.4) to the SSA. The Guided wSSA, by contrast, was around 25% as efficient as the SSA despite performing similarly on this model (Figure 2D).

Algorithm	μ_w	σ_w^2
SSA	0	0
wSSA	1.4753×10^{-7}	9.1059×10^{-13}
swSSA	6.2517×10^{-8}	1.0426×10^{-12}
Guided wSSA	0	0

Table 1: Estimated probability and run weight variance produce by each algorithm when simulating the enzymatic futile cycle for 103 runs (true probability $1.71 \times 10-7$). Near-optimal biasing parameters were used for the wSSA and swSSA ($\delta = 0.5$, γmax = 2.5).

We found that the wSSA estimated p more accurately and precisely than the swSSA. In 103 runs, the wSSA estimated p with 13.7% relative error while the swSSA estimated p with 63.4% relative error, indicating that the wSSA is more accurate. Likewise, the wSSA produced smaller run weight variance than the swSSA, indicating that the wSSA was also more precise. Given that the wSSA also performed with better runtime efficiency than the swSSA, it is clear that the state-dependent

biasing applied by the swSSA was not helpful when simulating the enzymatic futile cycle (Table 1, Figure 2D).

4.2 Modified Yeast Polarization

Next, we applied each IS algorithm to the modified yeast polarization model (Section 3.1.2) to determine the probability of Gbg reaching 50 or more molecules before 20 time units have passed. The modified yeast polarization model is a typical biochemical reaction network which is more complicated than the enzymatic futile cycle, so these results are reflective of each algorithm's ability to model rare events in more complicated biochemical systems. The probability of the event of interest in this model is $p = 1.202 \times 10{-}6$.

We again found that the wSSA and swSSA estimated p much faster than the SSA after 104 runs, but were sensitive to biasing parameters, while the Guided wSSA performed worse than the SSA. Biasing parameter optimization (Section 3.2.1) revealed that the wSSA and swSSA performed near optimally with biasing parameters $\delta = 0.0.725$ and $\gamma max = 20$, respectively. However, both algorithms exhibited high relative error and poor performance as their biasing parameters varied from these values (Figure 3B and C). There is no existing technique for deriving these optimal biasing parameters without first knowing p. Convergence comparison (Section 3.2.2 revealed that, with these near-optimal biasing parameters, the wSSA and swSSA estimated p well within 104 runs, while the SSA and Guided wSSA failed to reach the state of interest even once (Figure 3A). The enhanced run efficiency of the wSSA and swSSA relative to the SSA does indicate that the user may estimate p more rapidly with these methods because the wSSA and swSSA performed with similar runtime efficiency (Section 3.2.4) to the SSA. The Guided wSSA, by contrast, was around 33% as efficient as the SSA despite performing similarly on this

model (Figure 3D). This disparity is less severe than in the futile cycle case, indicating that Guided wSSA was less slow relative to the other algorithms on this model.

We found that the swSSA estimated p more accurately and precisely than the swSSA, in contrast to the futile cycle case. In 103 runs, the wSSA estimated p with 3592.5% relative error while the swSSA estimated p with 0.3% relative error, indicating that the swSSA is more accurate. Likewise, the wSSA produced larger run weight variance than the swSSA, indicating that the swSSA was also more precise. In this case, it is clear that the statedependent biasing applied by the swSSA was helpful when simulating the enzymatic futile cycle. These results indicate that the superior performance of the wSSA in the futile cycle model does not generalize (Table 2).
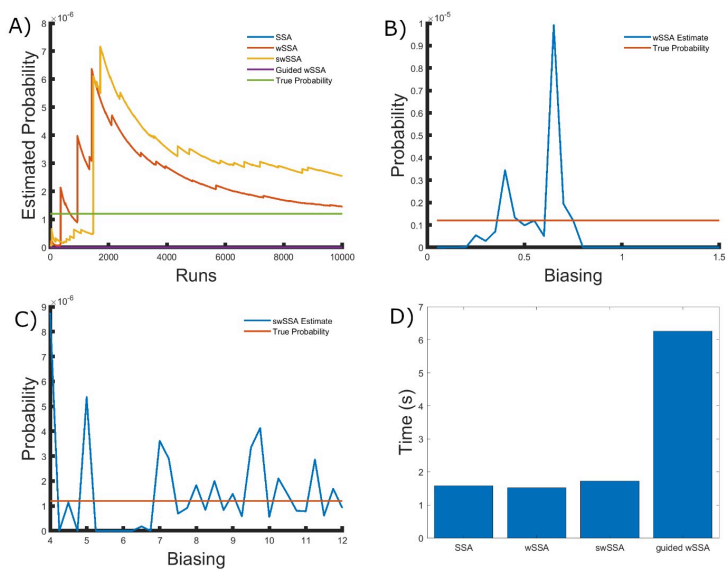
Figure 3: Simulation results for the modified yeast polarization model (true probability 1.202×10^{-6}). (A) Convergence of Monte Carlo estimated probability for the SSA,

wSSA, swSSA, and Guided wSSA over 104 runs (δ = 0.725, γmax = 20). (B) Probability estimated by the wSSA at 104 runs as a function of δ. (C) Probability estimated by the swSSA at 104 runs as a function of γmax. (D) Time for the SSA, wSSA, swSSA, and Guided wSSA to complete 103 runs.

4.3 Simplified Motility Regulation

Next, we applied each IS algorithm to the simplified motility regulation model (Section 3.1.3) to determine the probability of CodY reaching 20 or more molecules before 10 time units have passed. The simplified motility regulation model is a typical gene regulatory network, so these results are reflective of each algorithm's ability to model rare events in naturally occurring gene regulatory networks. The probability of the event of interest in this model is p = 2.161 × 10–7.

We found that the wSSA, swSSA, and Guided wSSA estimated p much faster than the SSA after 104 runs, but the wSSA and swSSA were sensitive to biasing parameters. This is the first model in which the Guided wSSA outperforms the SSA. Biasing parameter optimization (Section 3.2.1) revealed that the wSSA and swSSA performed near optimally with biasing parameters δ = 0.3 and γmax = 8.25, respectively. However, both algorithms exhibited high relative error and poor performance as their biasing parameters varied from these values (Figure 4B and C). There is no existing technique for deriving these optimal biasing parameters without first knowing p. Convergence comparison (Section 3.2.2 revealed that, with these near-optimal biasing parameters, the wSSA and swSSA estimated p well within 104 runs, and the Guided wSSA did so without any user-selected biasing.

Algorithm	μ_w	σ_w^2
SSA	0	0
wSSA	4.4384×10^{-5}	1.8610×10^{-6}
swSSA	1.2061×10^{-6}	4.7423×10^{-10}
Guided wSSA	0	0

Table 2: Estimated probability and run weight variance produce by each algorithm when simulating the modified yeast polarization model for 103 runs (true probability 1.202×10−6). Near-optimal biasing parameters were used for the wSSA and swSSA (δ = 0.725, γmax = 20).

The SSA again failed to reach the state of interest even once (Figure 4A). The enhanced run efficiency of the wSSA and swSSA relative to the SSA does indicate that the user may estimate p more rapidly with these methods because the wSSA and swSSA performed with similar runtime efficiency (Section 3.2.4) to the SSA. The Guided wSSA was around 33% as efficient as the SSA but did perform well on this model (Figure 4D). In this case, the Guided wSSA is well-suited to the problem of rare event simulation and eliminates the need for biasing parameter selection.

Algorithm	μ_w	σ_w^2
SSA	0	0
wSSA	6.3233×10^{-8}	6.4963×10^{-13}
swSSA	3.3120×10^{-8}	2.5388×10^{-13}
Guided wSSA	6.4949×10^{-7}	2.4402×10^{-10}

Table 3: Estimated probability and run weight variance produce by each algorithm when simulating the motility regulation model for 103 runs (true probability 2.161×10−7).

Near-optimal biasing parameters were used for the wSSA and swSSA ($\delta = 0.3$, γmax = 8.25).

We found that the wSSA estimated p more accurately and precisely than the swSSA or Guided wSSA. In 103 runs, the wSSA estimated p with 70.7% relative error while the swSSA estimated p with 84.7% relative error and the Guided wSSA estimated p with a 200.6% error, indicating that the wSSA is the most accurate. The Guided wSSA was also the least precise, though the swSSA was more precise than the wSSA despite being less accurate. This undermines the traditional use of run weight variance as a measure of IS scheme optimality. Though the wSSA and swSSA both performed better than the Guided wSSA in this case as they have in the previous two cases, the Guided wSSA does converge better than the SSA in this example. For this reason and because the Guided wSSA does not rely on user-selected biasing parameters, the Guided wSSA would be the safest option for this model, unlike in the previous two (Table 3).

4.4 Genetic Circuit 0x8E

Next, we applied each IS algorithm to the genetic circuit 0x8E model (Section 3.1.4) to determine the probability of Y FP reaching 70 or more molecules before 1000 time units have passed. The genetic circuit 0x8E model is a typical synthetic genetic circuit, so these results are reflective of each algorithm's ability to model rare events in synthetic genetic circuits. The probability of the event of interest in this model is $p = 6.29 \times 10{-4}$. We found that the SSA, wSSA, swSSA, and Guided wSSA all estimated p similarly after 104 runs, but the wSSA and swSSA were sensitive to biasing parameters and the Guided wSSA was inefficient. This is the first model in which the SSA performs well. Biasing parameter optimization (Section 3.2.1) revealed that the wSSA and swSSA performed

near optimally with biasing parameters δ = 0.6 and γmax = 4.5, respectively. However, both algorithms exhibited high relative error and poor performance as their biasing parameters varied from these values (Figure 5B and C). There is no existing technique for deriving these optimal biasing parameters without first knowing p. Convergence comparison (Section 3.2.2 revealed that, with these near-optimal biasing parameters, the SSA, wSSA and swSSA, and Guided wSSA all estimated p well within 104 runs, and the SSA and Guided wSSA did so without any user-selected biasing (Figure 5A). The SSA is the best suited to rare event simulation in this case due to its lack of user-selected biasing parameters and superior runtime efficiency (Section 3.2.4, Figure 5D).
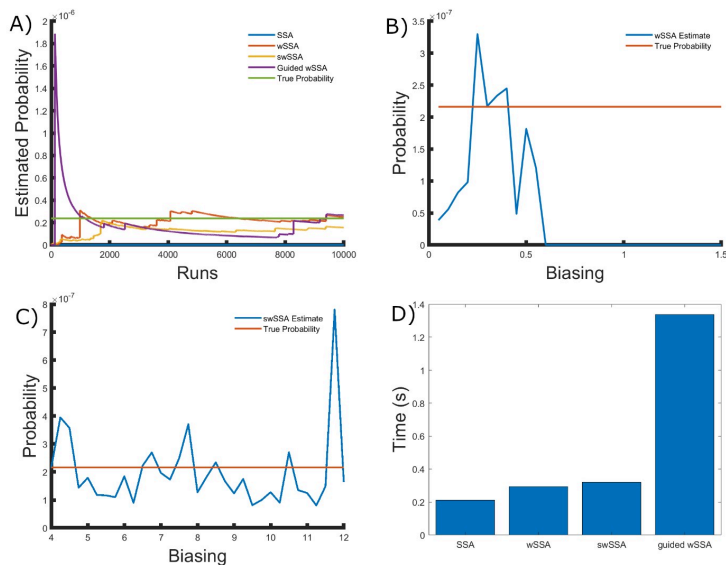
Figure 4: Simulation results for the simplified motility regulation model (true probability 2.161 × 10−7). (A) Convergence of Monte Carlo estimated probability for the SSA, wSSA, swSSA, and Guided wSSA over 104 runs (δ = 0.3, γmax = 8.25). (B) Probability estimated by the wSSA at 104 runs

as a function of δ. (C) Probability estimated by the swSSA at 104 runs as a function of γmax. (D) Time for the SSA, wSSA, swSSA, and Guided wSSA to complete 103 runs.

the SSA, wSSA and swSSA, and Guided wSSA all estimated p well within 104 runs, and the SSA and Guided wSSA did so without any user-selected biasing (Figure 5A). The SSA is the best suited to rare event simulation in this case due to its lack of user-selected biasing parameters and superior runtime efficiency (Section 3.2.4, Figure 5D). We found that the wSSA estimated p more accurately and precisely than the SSA, swSSA or Guided wSSA. In 103 runs, the wSSA estimated p with 20.4% relative error while the swSSA estimated p with 56.6% relative error and the Guided wSSA estimated p with a 99.6% error, indicating that the wSSA is the most accurate. As in the modified motility regulation model, the Guided wSSA was also the least precise, though the swSSA was more precise than the wSSA despite being less accurate. This again undermines the traditional use of run weight variance as a measure of IS scheme optimality (Table 4).

4.5 Genetic Circuit 0x8E LHF

Next, we applied each IS algorithm to the genetic circuit 0x8E LHF model (Section 3.1.5) to determine the probability of Y FP reaching 160 or more molecules before 1000 time units have passed. The genetic circuit 0x8E LHF model is a typical synthetic genetic circuit like 0x8E, so these results are reflective of each algorithm's ability to model rare events in synthetic genetic circuits. The probability of the event of interest in this model is $p = 7.33 \times 10^{-4}$.
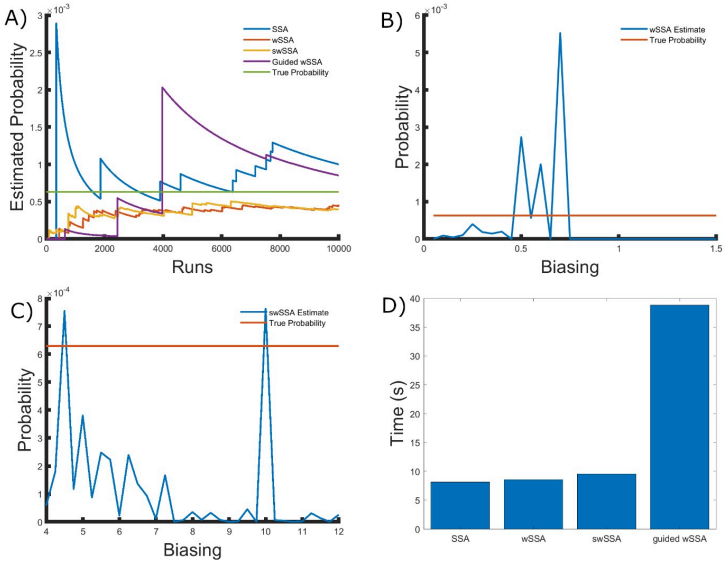
Figure 5: Simulation results for the genetic circuit 0x8E model (true probability 6.29 × 10−4). (A) Convergence of Monte Carlo estimated probability for the SSA, wSSA, swSSA, and Guided wSSA over 10^4 runs (δ = 0.6, γ_{max} = 4.5). (B) Probability estimated by the wSSA at 10^2 runs as a function of δ. (C) Probability estimated by the swSSA at 10^2 runs as a function of γ_{max}. (D) Time for the SSA, wSSA, swSSA, and Guided wSSA to complete 10^3 runs.

We found that the wSSA, swSSA, and Guided wSSA all estimated p similarly after 10^4 runs, but the wSSA and swSSA were sensitive to biasing parameters and the Guided wSSA was inefficient. Biasing parameter optimization (Section 3.2.1) revealed that the wSSA and swSSA performed near optimally with biasing parameters δ = 0.7 and γ_{max} = 1.75, respectively. However, both algorithms exhibited high relative error and poor performance as their biasing parameters varied from these values (Figure 6B and C). There is no existing technique for deriving these optimal biasing parameters without first

knowing p. Convergence comparison (Section 3.2.2 revealed that, with these near-optimal biasing parameters, the wSSA, swSSA, and Guided wSSA all estimated p well within 104 runs, and Guided wSSA did so without any user-selected biasing (Figure 6A). The Guided wSSA is the best suited to rare event simulation in this case due to its lack of user-selected biasing parameters, but is only around 33% as runtime efficient as the other methods (Section 3.2.4, Figure 6D). We found that the wSSA estimated p more accurately and precisely than the swSSA or Guided wSSA. In 103

Algorithm	μ_w	σ_w^2
SSA	0	0
wSSA	5.0043×10^{-4}	9.8086×10^{-5}
swSSA	2.7317×10^{-4}	2.8359×10^{-5}
Guided wSSA	2.7908×10^{-6}	7.7807×10^{-9}

Table 4: Estimated probability and run weight variance produce by each algorithm when simulating the genetic circuit Ox8E model for 10^3 runs (true probability 6.29×10^{-4}). Near-optimal biasing parameters were used for the wSSA and swSSA ($\delta = 0.6$, $\gamma_{max} = 4.5$).

Algorithm	μ_w	σ_w^2
SSA	0	0
wSSA	7.3761×10^{-4}	1.2681×10^{-5}
swSSA	6.0421×10^{-4}	9.7109×10^{-5}
Guided wSSA	7.5312×10^{-4}	1.7827×10^{-5}

Table 5: Estimated probability and run weight variance produce by each algorithm when simulating the genetic circuit Ox8E LHF model for 103 runs (true probability 7.33×10−4). Near-optimal biasing parameters were used for the wSSA and swSSA (δ = 0.7, γmax = 1.75).

runs, the wSSA estimated p with 0.6% relative error while the swSSA estimated p with 17.6% relative error and the Guided wSSA estimated p with a 2.7% error, indicating that the wSSA is the most accurate. This is the first model in which the Guided wSSA outperformed the swSSA. In this case, low run weight variance was indicative of a superior performance (Table 5).

4.6 Genetic Circuit 0x8E TI

Next, we applied each IS algorithm to the genetic circuit

0x8E TI model (Section 3.1.6) to determine the probability of Y FP reaching 100 or more molecules before 1000 time units have passed. The genetic circuit 0x8E TI model is a typical synthetic genetic circuit like 0x8E and 0x8E LHF, so these results are reflective of each algorithm's ability to model rare events in synthetic genetic circuits. The probability of the event of interest in this model is $p = 8.74 \times 10{-4}$.

We found that the SSA and wSSA estimated p well after 104 runs, but the wSSA was sensitive to biasing parameters. This is the first model in which the swSSA does not perform well with optimal biasing parameters. Biasing parameter optimization (Section 3.2.1) revealed that the wSSA and swSSA performed near optimally with biasing parameters $\delta = 0.85$ and $\gamma_{max} = 3.75$, respectively. However, both algorithms exhibited high relative error and poor performance as their biasing parameters varied from these values (Figure 6B and C). There is no existing technique for deriving these optimal biasing parameters without first knowing p. Convergence comparison (Section 3.2.2 revealed that, with these near-optimal biasing parameters, the SSA and wSSA estimated p well within 104 runs, and the SSA did so without any user-selected biasing (Figure 7A). The SSA is the best suited to rare event simulation in this case due to its lack of user-selected biasing parameters and its superior runtime efficiency (Section 3.2.4, Figure 7D).

We found that the wSSA estimated p more accurately than the swSSA or Guided wSSA, though the swSSA and Guided wSSA reported higher precision. In 103 runs, the wSSA estimated p with 2.8% relative error while the swSSA estimated p with 85.2% relative error and the Guided wSSA estimated p with a 90.6% error, indicating that the wSSA is the most accurate. If the user were using run weight variance as a metric

of optimality, they would fail to recognize the superior peroformance of the wSSA, however (Table 6).
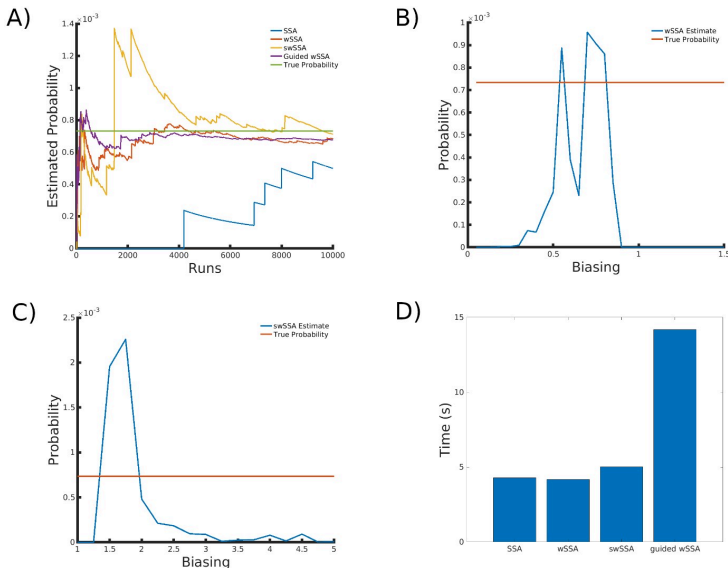
Figure 6: Simulation results for the genetic circuit 0x8E LHF model (true probability 7.33 × 10−4). (A) Convergence of Monte Carlo estimated probability for the SSA, wSSA, swSSA, and Guided wSSA over 104 runs (δ = 0.7, γ_{max} = 1.75). (B) Probability estimated by the wSSA at 102 runs as a function of δ. (C) Probability estimated by the swSSA at 102 runs as a function of γ_{max}. (D) Time for the SSA, wSSA, swSSA, and Guided wSSA to complete 103 runs.

4.7 Mathematical Analysis

We found that, across all our models, the wSSA and swSSA exhibited biasing parameter sensitivity and predominantly underestimated the true probability of the event of interest when given biasing parameters outside the narrow range of 'good' parameters. Likewise, the Guided wSSA tended to underestimate the probability of the event of interest when it did not perform well. To understand why this occurs, we

considered importance sampling schemes on a simple, abstract stochastic process.

Consider a process which being in state S0 and then transitions to some state in Ω = {S1, . . . , SN} with probability p1, . . . , pN, respectively. Denote this distribution as f. Let X be a random variable denoting the state the process enters after leaving S0 8. Suppose we want to know

$$p = P\{X \in E\}$$
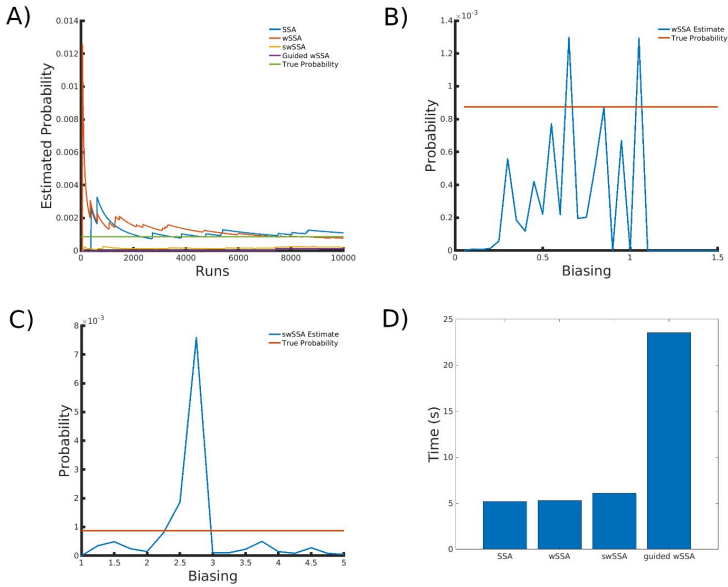
Figure 7: Simulation results for the genetic circuit 0x8E TI model (true probability 8.74×10−4). (A) Convergence of Monte Carlo estimated probability for the SSA, wSSA, swSSA, and Guided wSSA over 104 runs (δ = 0.85, γmax = 3.75). (B) Probability estimated by the wSSA at 103 runs as a function of δ. (C) Probability estimated by the swSSA at 103 runs as a function of γmax. (D) Time for the SSA, wSSA, swSSA, and Guided wSSA to complete 103 runs.

for some $E \subset \Omega$. The naive Monte Carlo approach is to approximate

$$p = E_f[I(x)] \approx \frac{1}{N} \sum_{n=1}^{N} I(x_n),$$

where X_n is distributed according to f, $E_f[\cdot]$ denotes the expected value under distribution f, and $I(x)$ is a binary indicator function:

$$I(x) = \begin{cases} 1 & x \in E \\ 0 & x \notin E \end{cases}.$$

This paradigm may be accelerated with importance sampling, let

$$g^*(S_k) = \begin{cases} \frac{f(S_k)}{p} & S_k \in E \\ 0 & \text{otherwise} \end{cases}$$

Algorithm	μ_w	σ_w^2
SSA	0	0
wSSA	8.9846×10^{-4}	4.2146×10^{-4}
swSSA	1.2945×10^{-4}	5.9258×10^{-6}
Guided wSSA	8.2357×10^{-5}	6.7493×10^{-6}

Table 6: Estimated probability and run weight variance produce by each algorithm when simulating the genetic circuit Ox8E_TI model for 10^3 runs (true probability 8.74×10^{-4}). Near-optimal biasing parameters were used for the wSSA and swSSA ($\delta = 0.85$, $\gamma_{max} = 3.75$).

be the importance density. Under g^*, we would approximate p as

$$p = E_g[I(x)w(x)] \approx \frac{1}{N} \sum_{n}^{N} I(x_n)w(x_n) = \sum_{n=1}^{N} I(x_n)\frac{f(x_n)}{f(x_n)/p} = \sum_{n=1}^{N} I(x)p,$$

where x_n is distributed according to g^*. However, because g^* is nonzero for only $x \in E$, each sample has $I(x_n) = 1$, so we find

$$\frac{1}{N} \sum_{n=1}^{N} I(x)p = p,$$

that is, the sample estimator under g∗ is always equivalent to p, so p may be exactly calculated with only one sample, and any set of samples with have a weight variance of zero. g∗ is therefore the ideal importance density [8]. Note that the ideal importance density is everywhere either zero or proportional to the distribution f. In practice, however, p is not known and it may be nonobvious which subset of the sample space E ⊂ Ω corresponds to a rare event of interest. Suppose instead we have the nonideal importance density.

$$g(S_k) = \begin{cases} \frac{f(S_k)}{p'} & S_k \in E' \\ 0 & \text{otherwise} \end{cases},$$

where $E' \subset E \subset \Omega$ and $p' = P\{X \in E'\}$. We would estimate p as

$$\frac{1}{N} \sum_{n=1}^{N} I(x_n)\frac{f(x_n)}{f(x_n)/p'} = p' < p$$

Note that, like the ideal importance density function g∗, the

nonideal g yields zero-variance weights. In reality, a nonideal importance density may be vanishingly small but nonzero for $x \in E - E'$ and may slightly deviate from $f(x)/p'$ for $x \in E'$. In this case, many samples must be drawn from g before a single $x \in E - E'$ is produced. When one is, it will have a large weight because $g(x) << f(x)$, which accounts for the exactness of importance sampling. However, before that sample is produced, the Monte Carlo estimator will yield an estimate very close to p' with small weight variance. If small weight variance is used as an indicator of importance density optimality, such an importance sampling scheme will appear optimal while consistently producing underestimates for p. This issue is not a feature of rare event simulation, but rather a feature of importance sampling itself. It arises in the context of rare event simulation, however, because IS-based algorithms are not necessary when the events of interest are not rare. Within the context of the stochastic simulation algorithms for chemical reaction networks presented here, poor importance densities are sure to arise. This is because the methods considered here bias individual reactions, rather than individual elements of the sample space, that is, paths. Within this framework the importance density cannot be zero for paths which do not reach the state(s) of interest, nor can all paths to the state(s) of interest have importance density proportional to their true probability. In particular, the methods presented here bias some reactions upward and bias some downward. This causes paths to the state(s) of interest which include reactions which have been biased downward to have vanishingly small importance density, which leads to severe underestimates in all but the most optimal biasing schemes (Figures 2B-C, 3B-C, 4B-C, 5B-C, 6B-C, 7B-C). Likewise, the methods presented here produce large importance densities at

paths which include many upward biased reactions but which do not reach the state of interest, which increases the number of zero-weight runs and delays estimator convergence further.
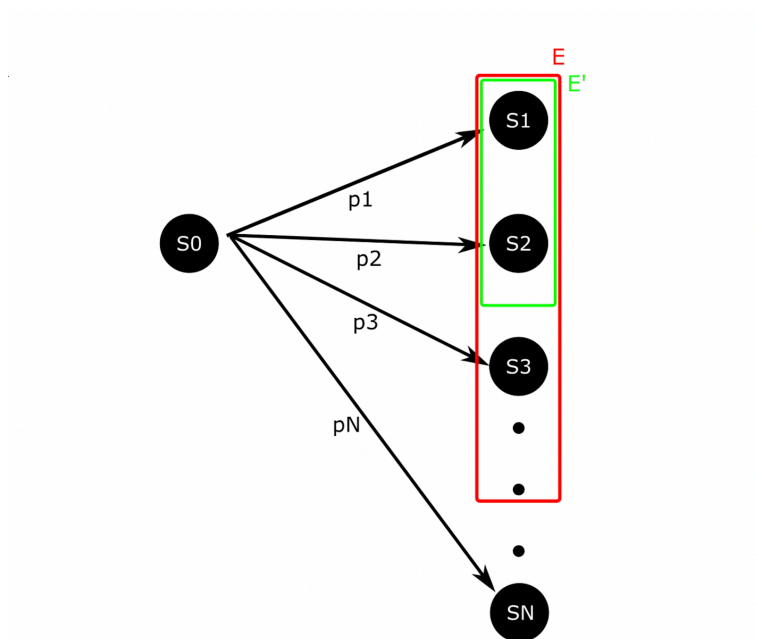
Figure 8: A simple, abstract stochastic process. Analysis of this process reveals the inherent challenges of ISbased algorithms.

5 Discussion

We implemented several existing stochastic simulation algorithms which make use of importance sampling (IS) to evaluate whether they were well-suited to the task of genetic circuit design verification. To this end, we developed a set of model biochemical systems case studies and algorithmic benchmarks which reflect the performance of an algorithmic in genetic circuit design. These analyses revealed that no existing algorithm is reliable enough to be used in design workflows.

We concluded that IS-based simulation must be further developed to accelerate genetic circuit design.

Our investigations revealed that the weighted stochastic simulation algorithm (wSSA) [9] was able to estimate low probabilities more rapidly than any other method when given optimal biasing parameters (Figures 2A, 3A, 4A, 5A, 6A, 7A). The wSSA was able to do this as quickly or faster than the traditional SSA (Figures 2D, 3D, 4D, 5D, 6D, 7D). However, the wSSA also exhibited biasing sensitivity in all cases (Figures 2B, 3B, 4B, 5B, 6B, 7B). There does not exist any method for determining optimal biasing parameters without already knowing the true probability of the event of interest, as in our case studies. This makes the wSSA impractical for verifying actual genetic circuit designs. When the wSSA was biased poorly, it tended to underestimate the probability of the event of interest (Figures 2B, 3B, 4B, 5B, 6B, 7B), which is consistent with our understanding of IS methods in general (Section 4.7). Due to biasing sensitivity, the wSSA is not reliable enough to be used in genetic circuit design verification.

Likewise, the state-dependent weighted stochastic simulation algorithm (swSSA) [10] was able to rapidly estimate low probabilities (though not as rapidly as the wSSA) with optimal biasing parameters for all case studies (Figures 2A, 3A, 4A, 5A, 6A, 7A). The swSSA was able to do this with only a minimal loss of efficiency relative to the traditional SSA (Figures 2D, 3D, 4D, 5D, 6D, 7D). Again, however, the algorithm exhibited sensitivity to biasing parameters and there exists no method of determining which biasing parameters will be optimal without a priori knowledge of the event of interest (Figures 2C, 3C, 4C, 5C, 6C, 7C). The swSSA exhibited more robustness than the wSSA for smaller models (Figures 2C, 3C, 4C), but performed similar to the wSSA for actual

genetic circuit designs (Figures 5C, 6C, 7C). The swSSA also underestimated probabilities when biased poorly, which is consistent with our understanding of IS methods in general (Section 4.7). Like the wSSA, the swSSA is too sensitive to biasing parameters to be used in genetic circuit design verification.

Unlike the wSSA and swSSA, the guided weighted stochastic simulation algorithm (Guided wSSA) [11] was not able to rapidly estimate the probability of rare events of interest in several of our case studies (Figures 2A, 3A, 7A). Despite its poor performance, the Guided wSSA also exhibited worse runtime efficiency than any other method, by a factor of around three (Figures 2D, 3D, 4D, 5D, 6D, 7D). The Guided wSSA does not rely on user-selected biasing parameters so it sidesteps the shortcomings of the wSSA and swSSA. However, the Guided wSSA is nonetheless too run-inefficient and time-inefficient to be suitable for genetic circuit design verification.

The wSSA, swSSA, and Guided wSSA each promised to solve the problem of rare event simulation in the verification of biochemical reaction networks in their respective publications [9–11]. We found this not to be the case. Previous work used run weight variance as a measure of biasing scheme optimality to show that IS methods perform well. However, we found that run weight variance is not suitable for that purpose (Tables 1, 2, 3, 4, 5, 6; Section 4.7). Additionally, The published description of the Guided wSSA is particularly misleading because modifications to the algorithm are required for it run stably and to replicated the published results (Section 2.3.3).

This work is limited, however, because each model was biasing using only one biasing factor, to simplify biasing factor optimization to a one-dimensional problem. The set of effective biasing schemes in higher dimensions may well vary in size

and shape from the one-dimensional case. We also considered only individual simulation traces for each algorithm and model. A more robust approach would be to generate many traces and report on summary statistics instead.

Efficient and robust methods of rapidly estimating the probability of rare failure states are crucial to the computer aided verification of genetic circuits. If such methods can be developed, the design phase of the designbuild- test-learn (DBTL) cycle will be strengthened and genetic circuits will be prototyped much more quickly. To achieve this, however, more work is necessary. Future work may include the development of an IS-based stochastic simulation algorithm that is both run-efficient and time-efficient like the wSSA and swSSA but which is not reliant on user input like the Guided wSSA. Alternatively, future work could develop a method of deriving near-optimal biasing factors to be used with the wSSA or swSSA, perhaps using machine learning techniques. Future workflows could also make use of IS-based methods like those presented here to establish likely lower bounds on probabilities. Establishing a lower bound could prove useful if used in conjunction with other model checking techniques, such as counterexample generation [18].

Armed with efficient rare event simulation methods, synthetic biologists could make fewer orbits around the DBTL cycle when developing genetic circuits for applications in biofuels, biomanufacturing, and medicine. This would enable rapid prototyping of genetic devices which would have the potential to greatly improve quality of life.

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References

[1] Ahmad S. Khalil and James J. Collins. Synthetic biology: applications come of age. Nature Reviews Genetics, 11(5):367–379, May 2010.

[2] Rongming Liu, Marcelo C. Bassalo, Ramsey I. Zeitoun, and Ryan T. Gill. Genome scale engineering techniques for metabolic engineering. Metabolic Engineering, 32:143–154, 2015.

[3] Michael S Samoilov and Adam P Arkin. Deviant effects in molecular reaction pathways. Nature biotechnology, 24(10):1235–1240, 2006.

[4] Pedro Fontanarrosa, Hamid Doosthosseini, Amin Espah Borujeni, Yuval Dorfan, Christopher A. Voigt, and Chris Myers. Genetic circuit dynamics: Hazard and glitch analysis. ACS Synthetic Biology, 9(9):2324–2338, 2020. PMID: 32786351.

[5] Lukas Buecherl, Riley Roberts, Pedro Fontanarrosa, Payton J. Thomas, Jeanet Mante, Zhen Zhang, and Chris J. Myers. Stochastic hazard analysis of genetic circuits in ibiosim and stamina. ACS Synthetic Biology, 10(10):2532–2540, 2021. PMID: 34606710.

[6] Daniel T Gillespie. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. Journal of Computational Physics, 22(4):403–434, 1976.

[7] Daniel T. Gillespie. Exact stochastic simulation of coupled chemical reactions. The Journal of Physical Chemistry, 81(25):2340–2361, 1977.

[8] Surya T. Tokdar and Robert E. Kass. Importance

sampling: a review. WIREs Computational Statistics, 2(1):54–60, December 2009.

[9] Hiroyuki Kuwahara and Ivan Mura. An efficient and exact stochastic simulation method to analyze rare events in biochemical systems. The Journal of chemical physics, 129(16):10B619, 2008.

[10] Min K Roh, Dan T Gillespie, and Linda R Petzold. State-dependent biasing method for importance sampling in the weighted stochastic simulation algorithm. The Journal of chemical physics, 133(17):174106, 2010.

[11] Colin S Gillespie and Andrew Golightly. Guided proposals for efficient weighted stochastic simulation. The Journal of chemical physics, 150(22):224103, 2019.

[12] Andrew Golightly and Darren JWilkinson. Bayesian inference for markov jump processes with informative observations. Statistical applications in genetics and molecular biology, 14(2):169–188, 2015.

[13] Timothy S. Gardner, Charles R. Cantor, and James J. Collins. Construction of a genetic toggle switch in escherichia coli. Nature, 403(6767):339–342, January 2000.

[14] Michael B. Elowitz and Stanislas Leibler. A synthetic oscillatory network of transcriptional regulators. Nature, 403(6767):335–338, January 2000.

[15] A. A. K. Nielsen, B. S. Der, J. Shin, P. Vaidyanathan, V. Paralanov, E. A. Strychalski, D. Ross, D. Densmore, and C. A. Voigt. Genetic circuit design automation. 352(6281). 26

[16] Ophir Flomenbom, Kelly Velonia, Davey Loos, Sadahiro Masuo, Mircea Cotlet, Yves Engelborghs, Johan Hofkens, Alan E Rowan, Roeland JM Nolte, Mark Van der Auweraer, et al. Stretched exponential decay and correlations in the catalytic activity of fluctuating single lipase molecules. Proceedings of the National Academy of Sciences, 102(7):2368–2372, 2005.

[17] Brian Drawert, Stefan Hellander, Michael Trogdon, Tau-Mu Yi, and Linda Petzold. A framework for discrete stochastic simulation on 3d moving boundary domains. The Journal of Chemical Physics, 145(18):184113, 2016.

[18] Mohammad Ahmadi, Zhen Zhang, Chris Myers, Chris Winstead, and Hao Zheng. Counterexample generation for infinite-state chemical reaction networks, 2022.

Appendices

A   Pseudocode Algorithms

Algorithm 1 SSA

1: **for** $k = 1$ to n **do**
2:     $q \leftarrow 0$
3:     $t \leftarrow 0$
4:     $\mathbf{x} \leftarrow \mathbf{x}_0$
5:     evaluate all $a_j(\mathbf{x})$ and calculate $a_0(\mathbf{x})$
6:     **while** $t \leq t_{max}$ **do**
7:         **if** $x \in E$ **then**
8:             $q \leftarrow q + 1$
9:             Break out of while loop
10:        **end if**
11:        $\tau \leftarrow$ a sample of exponential variable with mean $1/a_0(\mathbf{x})$
12:        $u \leftarrow$ a sample of unit uniform random variable
13:        $\mu \leftarrow$ smallest integer satisfying $\sum_{i=1}^{\mu} \geq ub_0(\mathbf{x})$
14:        $t \leftarrow t + \tau$
15:        $\mathbf{x} \leftarrow \mathbf{x} + \nu_\mu$
16:        update $a_j(\mathbf{x})$ and recalculate $a_0(\mathbf{x})$
17:     **end while**
18: **end for**
19: report q/n as the estimated probability

Algorithm 2 wSSA

1: $q \leftarrow 0$
2: **for** $k = 1$ to n **do**
3:     $w \leftarrow 1$
4:     $t \leftarrow 0$
5:     $\mathbf{x} \leftarrow \mathbf{x}_0$
6:     evaluate all $a_j(\mathbf{x})$ and $b_j(\mathbf{x})$, and calculate $a_0(\mathbf{x})$ and $b_0(\mathbf{x})$
7:     **while** $t \leq t_{max}$ **do**
8:         **if** $x \in E$ **then**
9:             $q \leftarrow q + w$
10:            Break out of while loop
11:        **end if**
12:        $\tau \leftarrow$ a sample of exponential variable with mean $1/a_0(\mathbf{x})$
13:        $u \leftarrow$ a sample of unit uniform random variable
14:        $\mu \leftarrow$ smallest integer satisfying $\sum_{i=1}^{\mu} \geq ub_0(\mathbf{x})$
15:        $w \leftarrow w \times (a_\mu(\mathbf{x})/b_\mu(\mathbf{x})) \times (b_0(\mathbf{x})/a_0(\mathbf{x}))$
16:        $t \leftarrow t + \tau$
17:        $\mathbf{x} \leftarrow \mathbf{x} + \nu_\mu$
18:        update $a_j(\mathbf{x})$ and $b_j(\mathbf{x})$, and recalculate $a_0(\mathbf{x})$ and $b_0(\mathbf{x})$
19:     **end while**
20: **end for**
21: report q/n as the estimated probability

Algorithm 3 swSSA

1: Partition all reactions into three groups: G_E, G_D, G_N
2: **for** all $R_j \in G_D$ **do**
3:      choose $\rho_j^0 \in [0.1, 0.2]$
4:      choose the initial value for γ_j^{max}
5: **end for**
6: **for** all $R_j \in G_E$ **do**
7:      choose $\rho_j^0 \in [0.5, 0.6]$
8:      choose the initial value for γ_j^{max}
9: **end for**
10: **for** all $R_j \in G_N$ **do**
11:      $\gamma_j = 1 \quad \forall t, \, b_j(t) = a_j(t)$
12: **end for**
13: $q \leftarrow 0$
14: **for** $k = 1$ to n **do**
15:      $w \leftarrow 1$
16:      $t \leftarrow 0$
17:      $\mathbf{x} \leftarrow \mathbf{x}_0$
18:      evaluate all $a_j(\mathbf{x})$ and calculate $a_0(\mathbf{x})$
19:      calculate $\rho_j(\mathbf{x})$ for all $R_j \in G_E$ and all $R_j \in G_D$
20:      calculate all $\gamma_j(\mathbf{x})$; evaluate $b_j(\mathbf{x})$ and calculate $b_0(\mathbf{x})$
21:      **while** $t \leq t_{max}$ **do**
22:          **if** $x \in \varepsilon$ **then**
23:              $q \leftarrow q + w$
24:              Break out of while loop
25:          **end if**
26:          $\tau \leftarrow$ a sample of exponential variable with mean $1/a_0(\mathbf{x})$
27:          $u \leftarrow$ a sample of unit uniform random variable
28:          $\mu \leftarrow$ smallest integer satisfying $\sum_{i=1}^{\mu} \geq u b_0(\mathbf{x})$
29:          $w \leftarrow w \times (a_\mu(\mathbf{x})/b_\mu(\mathbf{x})) \times (b_0(\mathbf{x})/a_0(\mathbf{x}))$
30:          $t \leftarrow t + \tau$
31:          $\mathbf{x} \leftarrow \mathbf{x} + \nu_\mu$
32:          update $a_j(\mathbf{x})$ and $\rho_j(\mathbf{x})$
33:          recalculate $\gamma_j(\mathbf{x})$, update $b_j(\mathbf{x})$ and recalculate $b_0(\mathbf{x})$
34:      **end while**
35: **end for**
36: report q/n as the estimated probability

Algorithm 4 Guided wSSA

1: $q \leftarrow 0$
2: **for** $k = 1$ to n **do**
3:      $w \leftarrow 1$
4:      $t \leftarrow 0$
5:      $\mathbf{x} \leftarrow \mathbf{x_0}$
6:      $\Delta t \leftarrow t_{max}$
7:      evaluate all $a_j(\mathbf{x})$ and calculate $a_0(\mathbf{x})$
8:      **while** $t \leq t_{max}$ **do**
9:          **if** $\mathbf{x'} = F^T\mathbf{x}$ **then**
10:             $q \leftarrow q + w$
11:             Break out of while loop
12:          **end if**
13:          evaluate all d_i as $d_i = e_i(\mathbf{x_t}) - \mathbf{x'_i}) \times \mathrm{sgn}(\mathbf{x'_i} - [F^T\mathbf{x_0}]_i), \quad i = 1, \dots, u_0$
14:          **if** all $d_i > 0$ **then**
15:             $\mathbf{b(x)} \leftarrow \mathbf{a(x)}$ for all j.
16:          **else**
17:             $\mathbf{b(x_t)} \leftarrow \mathbf{a(x_t} + H(\mathbf{x_t})S^T F(F^T S H(\mathbf{x_t} S^T F \Delta t)^{-1} \times (\mathbf{x'} - F^T[\mathbf{x_t} + S\mathbf{a(x_t)}\Delta t])$
18:          **end if**
19:          $\tau \leftarrow$ a sample of exponential variable with mean $1/a_0(\mathbf{x})$
20:          $u \leftarrow$ a sample of unit uniform random variable
21:          $\mu \leftarrow$ smallest integer satisfying $\sum_{i=1}^{\mu} b_j(\mathbf{x} \geq ub_0(\mathbf{x})$
22:          $w \leftarrow w \times (a_\mu(\mathbf{x})/b_\mu(\mathbf{x})) \times \exp[b_0(\mathbf{x}) - a_0(\mathbf{x})]\tau$
23:          $t \leftarrow t + \tau$
24:          $|\Delta t \leftarrow t' - t$
25:          $\mathbf{x} \leftarrow \mathbf{x} + S^\mu$
26:          update $\mathbf{a(x)}$ and calculate a_0
27:      **end while**
28: **end for**
29: report q/n as the estimated probability

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28. **Research Reflection by Payton Thomas**

Payton Thomas

Faculty Mentor: Chris Myers (Electrical & Computer Engineering, University of Utah)

As an undergraduate student, I was involved in research throughout my degree program in Biomedical Engineering and Applied Mathematics, with a minor in Chemistry. I started working in a lab at the age of 16 and committed myself to a career in research. Over the course of my degree program, I collaborated with colleagues at several institutions, including Utah, USU, USF, and CU Boulder, and also had the opportunity to do research as a visiting scholar at MIT. During my undergraduate research, I mathematically analyzed algorithms that make use of importance sampling to estimate the probability of rare failure states in genetic circuits. Through my analysis, I found that these algorithms were not working as well as they were supposed to in their respective publications.

This research is important because rare failure states can have catastrophic consequences in biology, such as causing cancerous phenotypes. One of the biggest impacts of my undergraduate research experience was that it allowed me to develop strong skills in computational and theoretical research, as well as interdisciplinary collaboration. Research has been central in my education, as I pursued my degrees with an eye toward their relevance to my research fields. Additionally, my research experience taught me how to develop novel ideas from literature reviews and lead research projects on my own, which will be important in my future career as a grad student and beyond. My career aspirations were always to become a professor, and undergraduate research did not change that. Rather, it made me feel much more like a member of the department and academic community. Through my undergraduate research, I became a more competent computational and theoretical researcher, and I look forward to bringing these skills together as I work in the wet lab and continue my mathematical and computational research during my PhD program. Looking ahead, my major research goal is to develop models of synthetic genetic circuits using stochastic processes and dynamical systems. I hope to accelerate genetic circuit design to help solve significant medical problems such as cancer and aging. Through my research, I aim to have an impact on my fields and pursue a career as a professor, mentoring future scientists to advance the field of synthetic biology. Overall, my undergraduate research experience has been invaluable in shaping my education and future goals, and I am excited to continue to make important contributions to the field of synthetic biology and beyond.

About the Author

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29. **Design and Optimization of 3D Printed Pressure Sensors**

Derrick Wong

Faculty Mentor: Yong Lin Kong (Mechanical Engineering, University of Utah)

Total joint arthroplasty (TJA), or joint replacement surgery, is the most common solution for end-stage arthritis patients no longer responding to non-surgical treatment. From 2020 to 2021, the American Joint Replacement Registry reported 2.4 million total joint replacement surgeries for the hip and knee joints alone. However, failure rates for TJA joint implants as reported by the American Association of Hip and Knee Surgeons have been found to be 5-10% 10 years post-operatively. Common factors for failure include fracturing, implant instability, and biocompatibility concerns. However, there is currently no viable solution for implantable devices

that allows for monitoring in vivo. Here we fabricated wireless pressure sensors using additive manufacturing (3D printing), and showed that printed sensors yield a measurable signal in frequency space that may be characterized for future application with implantable devices.

For this project, we designed sensors using SOLIDWORKs, simulated them to better understand their response in frequency space, and measured successful prints using a network analyzer for comparison of simulated and observed resonant frequencies. In the span of this project, five design cycles were performed to iteratively optimize the sensor design. Each design involved changes to the geometry of the previous design, adjusting inductance and capacitance to obtain a readable resonant frequency and to shift the observed frequency range. The findings from these design optimizations provide insight into adjustments necessary to shift sensor resonant frequency into a desired range and highlights the importance of Q-factor in resonant frequency readability. These findings may also be referenced for a finalized design used in compression testing and characterization. We anticipate that the integration of 3D printed pressure sensors has potential for future use in wireless internal monitoring. The use of additive manufacturing for pressure sensors offers flexibility in fabrication and design geometry, which we envision will be useful in various implantable biomedical devices such as blood pressure monitors and joint implants.

About the Author

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30.   Research Reflection by Derrick Wong

Derrick Wong

Faculty Mentor: Yong Lin Kong (Mechanical Engineering, University of Utah)

With my research in the Additive Manufacturing Laboratory, I feel that I have greatly expanded on my personal hobby of 3D-design while also gaining a new confidence in my ability to formulate hypotheses. Under Dr. Kong and graduate student mentors, I truly learned the power of systematic troubleshooting and patience in overcoming the many challenges of my work. In addition to developing many skills and virtues necessary in biomedical engineering, my research in the past couple years has spurred my interest in biomedical device research and improving health outcomes. I am confident that the skills I have acquired with my work will serve as a strong foundation as I move on to pursue a degree in medicine and continue to conduct research.

About the Author

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31.   Electrical Impedance Dermography as a Biomarker for Non-Melanoma Skin Cancer

Elaine Wen Ying Wong;
Benjamin Sanchez
(Electrical and Computer
Engineering); and Douglas
Grossman (Huntsman
Cancer Institute)

Faculty Mentor: Benjamin Sanchez (Electrical and Computer Engineering, University of Utah)

Abstract: Diagnosis of basal cell and squamous cell carcinoma subtypes is difficult due to being unable to know

how deep the cancerous tissue goes. Therefore, different subtypes ideally require different types of biopsies, but due to being unable to visually see how deep the cancerous tissue goes, even expert dermatologists struggle to make this decision. Therefore, we hypothesize that using electrical impedance dermography can serve as a biomarker to distinguish these subtypes. After gathering data from real patients, we were able to find the area under curves of over 80% in all comparisons. that electrical impedance dermography will be able to serve as a biomarker for non-melanoma skin cancers.

Introduction: Clinical diagnosis of basal cell (BCC) and squamous cell (SCC) carcinoma subtypes is challenging. Multiple subtypes of BCC and SCC can be difficult to distinguish clinically and ideally require different biopsy techniques for optimal histologic analysis and therapeutic decision-making [1]. Visual detection of BCC and SCC can be facilitated with the aid of dermoscopy [2] but determining prior to biopsy whether a lesion is superficial or more deeply invasive is usually not possible. There is a great clinical need to develop new technologies to augment visual skin examination to guide biopsy-decision-making and improve the management of lesions suspicious for BCC and SCC. To date, there is no bedside technique available that is low-cost, easily applied, quantitative, objective, and capable of overcoming these diagnostic hurdles. EID is a newer non-invasive, quantitative, and objective tool sensitive enough to detect alterations in the electrical properties of skin cancers. The overarching hypothesis of my proposal is that EID can be used to distinguish BCC subtypes and between SCC-in situ, invasive SCC, and inflamed keratosis that cannot be appreciated clinically. Clinical Diagnosis of BCC and SCC subtypes is challenging.

The "superficial" form of BCC is confined to the epidermis and can be effectively treated by non-surgical means. The "nodular" form of BCC consists of a collection of round tumor cells occupying the upper part of the dermis and can be treated by destruction or surgically depending on its size and location. "Micronodular" and "infiltrative" forms of BCC consist of smaller aggregates of tumor cells or angulated or stranded tumor cells, respectively, infiltrating the deeper dermis and usually requiring surgical treatment. Importantly, these invasive subtypes of BCC can present as papules or plaques that cannot reliably be distinguished clinically from nodular or the more superficial subtype of BCC. While the "shave" biopsy technique is more appropriate for superficial BCC, the "punch" biopsy is preferred for invasive subtypes. The superficial form of SCC can resemble BCC, and it is challenging clinically to distinguish this entity from invasive SCC; the former is best biopsied by shave technique and can be effectively treated non-surgically while the latter is best biopsied by punch technique to assess the depth of invasion and usually requires surgical excision [3]. These histologic changes cannot be reliably appreciated visually, and thus distinguishing subtypes of BCC and SCC presents a clinical conundrum. EID technology could contribute to overall clinical assessment by increasing confidence and diagnostic accuracy that will inform biopsy-decision making in patients with lesions suspicious of skin cancer.

Data Gathering:

Using the innovative EID device as seen in Figure 1 and in collaboration with Dr. Douglas Grossman at the Huntsman Cancer Institute we gathered 17 BCC and 35 SCC lesions. Dr. Douglas Grossman is a Professor of Dermatology and Co-Leader of the Melanoma Center at the Huntsman Cancer

Institute. He has seen patients for skin cancer screening and treatment for the past 25 years and has extensive experience in the use of non-invasive technologies.

Figure 1. URSKIN handheld electrical impedance dermography device (A) Plot use of the device in the clinic (B) The reduced dimensions allow the operator to hold the device with one hand while with the other hand controls the device with a smartphone.
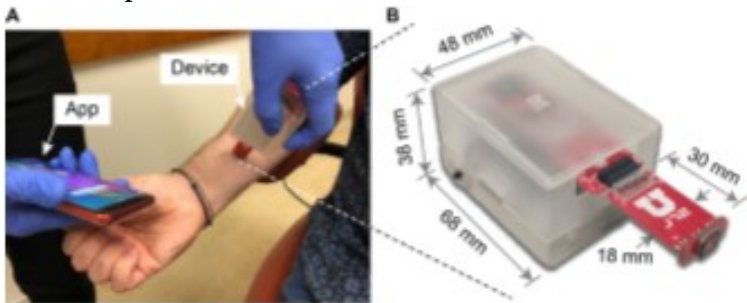
Using Machine Learning to Classify Non-Melanoma Skin Cancers: Using R-Studio we trained a machine learning random forest algorithm, combining EID data with morphologic and histologic characteristics and tumor subtype, using the bootstrapping sampling method to reduce overfitting. We split our data into two parts for the learning algorithm: 80% assigned to the training data, and 20% assigned to the test set. We then performed a nested 10-fold cross-validation approach for prediction with random forest in two loops. An inner loop will be allocated to determine the individual training data estimates and their performance, whereas the outer loop will be used for checking the ability of these estimates in making predictions on the test set. Combining the performance of each test set from all the outer loops will provide the final evaluation output of the learning algorithm. Finally, we will create receiver operator characteristics curves to check our algorithm's

performance for each of the grouped datasets, with the area under the curve providing the probability of the given learning model's ability to correctly classify.

Results:

My results showed EID to be very effective and efficient at diagnosing BCC and SCC. In the BCC study, I obtained a specificity of 88%. Similarly, in the SCC study, I achieved an averaged area under the curve of 0.968, a sensitivity of 94.6%, and a specificity of 96.9% (Fig.1). In both cases, my results exceed the diagnostic accuracy of using dermoscopy, the clinical gold standard technology. I currently am the first author of the pilot manuscript under review at the Journal of Investigative Dermatology Innovations [4] describing the results of my work.

Additional results showed the comparisons of SCC in situ versus normal skin which had an area under the curve of 82.5%. Invasive SCC vs normal skin is 95.5% and invasive SCC vs SCC in situ is 99.9%.

Discussion and Conclusion:

These results, even with small datasets, have already shown promising results for bioimpedance as a biomarker for non-melanoma skin cancers. This will only improve with increased dataset size.

This research will advance medical and scientific fields by establishing the connection between histological and electrical properties of skin cancer lesions. My work will enable rapid and accurate diagnosis of skin cancers and their subtypes, which currently rely heavily on dermatologist expertise and open opportunities for this relationship to be used to diagnose both malignant and benign lesions non-invasively. EID could be applied to other dermatology applications beyond skin

cancer detection including monitoring the effectiveness of treatment in individual patients and therapeutic research.

By establishing the histological-electrical relationship between healthy and diseased skin tissue, any person with a skin lesion can be diagnosed rapidly and accurately. My research will increase our understanding of electrical behavior in cancers non-invasively. My research will then be used to teach students translational applications of electrical engineering concepts to help people with medical conditions.

Future work for this research will include gathering more datasets for the SCC and BCC subtypes to make our machine-learning algorithms even more robust. Additional information will be gathered as well such as the specificity, sensitivity, and accuracy for each of the two-category measurements. Additionally, to improve its usage in the clinical environment the device and app that is being used would be updated to include the prediction resulting from the machine learning to give real-time predictions to the clinician.

Figure 2. (A) Receiver operator curve and (B) Confusion matrix of Machine Learning algorithm diagnosing SCC insitu and inflamed SK. Data from Wong et al., (4).
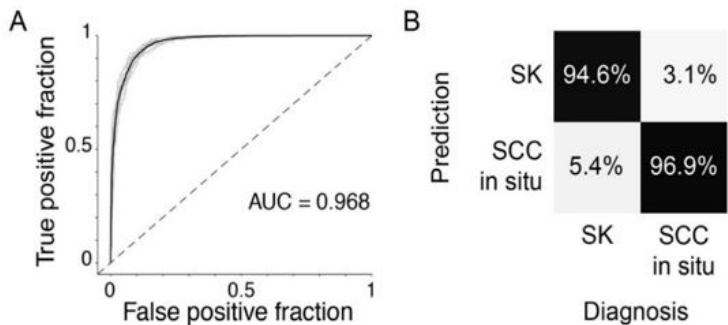
References:

[1] Cameron, M. C. et al. J Am Acad Dermatol 80, 321–339 (2019).

[2] Navarrete-Dechent, C. et al. JAMA Dermatol 156, 882 (2020).

[3] Motley, R. et al. British Journal of Dermatology 146, 18–25 (2002).

[4] Wong et al. Journal Investigative Dermatology Innovations, 2023.

About the Authors

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32. **Research Reflection by Elaine Wong**

Elaine Wen Ying Wong

Faculty Mentor: Benjamin Sanchez (Electrical and Computer Engineering, University of Utah)

I learned a lot about how to use electrical engineering concepts and apply them to the medical field. I also learned a lot about machine learning and how to get it to solve problems where we may want a more objective result than a subjective one. This has significantly impacted my education and future goals because this is the subject I will be researching in my doctorate program.

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SECTION V

College of Fine Arts

33. **Queering the Pipe Organ**

Samuel Judd

Faculty Mentor: Haruhito Miyagi (School of Music, University of Utah)

The overrepresentation of queer organists (especially gay men) in the United States pipe organcommunity has long been anecdotally acknowledged. While a causal relationship is hard toestablish, American narratives of the pipe organ over the past century can offer insight into therelationship between the pipe organ and queerness. I demonstrate that the history of the pipeorgan in the United States is laden with queerness in fictional portrayals, through its associationswith camp developments, and as a result of its relationship to the closet. The pipe organ hasgarnered associations with flamboyance, camp, fastidiousness, and secrecy, all of which bear aclose relationship to queerness. These patterns are nuanced by the pipe organ's association withChristian churches, pointing to a picture of the pipe organ's queerness as subversive in someAmerican liturgical settings. Following a queer

musicological approach, I argue that the pipeorgan has been uniquely coded through American cultural associations as a queer instrument, theimplications of which I discuss.

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SECTION VI

College of Health

34. Hip Mechanics as a Reflection of Physical Function in Aging Adults with Early to Late-Stage Hip Osteoarthritis

Hunter Blake Carlson and
Jesse C. Christensen
(Physical Therapy &
Athletic Training)

Abstract

Osteoarthritis is the most common joint disease in the world.

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[12] Its prevalence in the hip joint is observed to have dramatic adverse effects on the quality of life of aging adults and can contribute to more significant health issues. [1] Hip osteoarthritis (HOA) results in progressive joint pain and muscle loss, abnormal hip mechanics, and poor functional performance. [4, 8] To date, it is unknown how the abnormal hip mechanics brought on by HOA relate to functional performance. Therefore, this study aims to compare the relationship between deficits in hip mechanics of the frontal (primary) and sagittal (secondary) planes onto physical function, using validated functional performance tests in adults with HOA. We hypothesized that more significant deficits in the involved limb's hip mechanics (angle, moment, power) would be associated with poorer Two Minute Walk Test (2MWT) and Forty Meter Walk Test (40MWT) performance. We also hypothesized that more significant deficits in hip mechanics would be associated with greater hip abduction, knee extension, and knee flexion weakness of the involved limb. This hypothesis was explored, but no significant differences were found during the study. The results of this study demonstrated the following: 1) slower gait speeds were associated (p= 0.04, r= -0.47) with greater hip flexion angle in the late stance phase of the involved limb during self-selected walking; 2) faster gait speeds were associated (p= 0.04, r= 0.47) with greater hip power output in the early stance phase of the involved limb during self-selected walking; 3) more distance covered during the 2MWT was associated (p= 0.02, r= -0.53) with greater hip flexion angle in the late stance phase of the involved limb during self-selected walking; 4) more distance covered during the 2MWT was associated (p= 0.05, r= 0.44) with greater hip power in the late stance phase of the involved limb during self-selected walking; 5) slower 40MWT times

were associated (p< 0.01, r= -0.66) with greater hip flexion angle in the late stance phase of the involved limb during self-selected walking. No relationships between muscle strength and functional performance were observed. These findings demonstrate that adaptations in walking gait patterns of adults with HOA affect their overall functional performance, which could influence their quality of life and ability to participate in activities of daily living. Future studies can look at other planes of motion to attempt to build a more complete profile of the functional adaptations arising ii from HOA. This more complete profile can be used to address adults' concerns, improve quality of life metrics, and offset functional disability that is influenced by HOA.

Introduction

Osteoarthritis is the most common joint disease, affecting approximately 32 million adults within the United States, and contributes to nearly $140 billion/year in healthcare costs needed to manage this debilitating disease. [19, 29] The hip joint is one of the most common joints affected by the disease leading to debilitating joint pain and functional limitations. [1, 9, 14, 24] Hip osteoarthritis (HOA) disproportionately affects older adults 75 years of age and older and relates to a greater risk of a sedentary lifestyle contributing to an increased risk of health-related comorbidities. Additionally, HOA has been shown to increase age-adjusted mortality by 20 percent. [12] Adults with HOA demonstrate poor hip mechanics with tasks such as walking and transferring in/out of chairs. As it pertains to walking, adults with HOA demonstrate slower walking speed, shorter step length, and longer stance time on the non-involved limb. [3,4] Regarding transferring in/out of chairs, adults with HOA demonstrate a reduced hip range of motion and greater reliance on the non-involved limb when compared

to healthy-matched peers. [8] Additionally, adults with HOA demonstrate decreased physical function, muscle strength, and atrophy related to the disuse of the involved limb. More specifically, hip abduction, knee extensor, and knee flexor strength are significantly reduced in the HOA-affected limb relative to a healthy limb. Cross-sectional areas of the quadriceps, hamstrings, and gluteal muscles have shown significant reduction when compared to the healthy limb or healthy-matched peers. Adults with HOA have also been shown to have poorer functional performance on the Two Minute Walk Test (2MWT) and 30-Second Sit-to-Stand Test when compared to healthy peers. [6, 8]

The current strategies for managing HOA include exercise, weight management, braces, nonsteroidal anti-inflammatory drugs, topical treatments, and corticosteroid injections. [12, 13, 18] These methods are implemented to decrease pain and improve mobility brought on by the HOA-affected limb. When managing HOA becomes too difficult, or pain levels are too high to tolerate, total hip arthroplasty is the recommended strategy. Unfortunately, as hip pain is commonly resolved following surgery, adults still present with poor hip mechanics and functional performance. Ultimately, these impairments can result in reduced physical function and increased risk for sedentary health behavior even after the HOA pain is resolved.

Therefore, this study aims to compare the relationship between deficits in hip mechanics of the frontal (primary) and sagittal (secondary) planes onto physical function, using validated functional performance and muscle strength metrics in adults with HOA. We hypothesized that greater deficits in the involved limb's hip mechanics (angle, moment, power) would be associated with poorer 2MWT and Forty Meter Walk Test (40MWT) performance. We also hypothesized that greater

deficits in hip mechanics would be associated with greater hip abduction, knee extension, and knee flexion weakness of the involved limb.

Methods

This project involved bringing adults with late-stage HOA into the marker-based Gait Laboratory at the VA Salt Lake Health Care System. Adults completed several validated gait tests of 2MWT and 40MWT. Data collected this way was compared and processed via Nexus 2.1.1 software (Vicon, Oxford Metrics Ltd., Oxford, UK) and Visual3D (C-motion, Inc., Germantown, MD, USA). A cohort of 20 adults was used in this quality improvement project, with their pre-operative data collected 1-2 weeks before undergoing total hip replacement due to late-stage HOA.

All adults with HOA underwent testing at the VA Gait Laboratory using instrumented walkway with 12 embedded force plates (Bertec, Columbus OH, USA) and 24 infrared cameras (Nexus, Centennial CO, USA). Adults with HOA will be asked to 1) walk on a 5-meter 4 walkway, and we will analyze ten clean foot strikes between both limbs on the embedded force plates and 2) perform the 2MWT and 3) perform the 40MWT to compare hip mechanics of affected and nonaffected HOA limbs.

All adults were told the following basic script for the 2MWT:

1. We are going to have you walk for two minutes. We will have a timer counting down, so you do not need to worry about how much time is left in the test.

2. The goal is to walk as far as possible within the two-minute window. There is no need to achieve a specific distance. Walk at a speed that is comfortable and manageable for you.

3. We will do two practice walk tests before we collect the test trial. This will allow us to ensure that everything is working correctly and that you are not in any pain.

4. If you need to stop the trial at any point, please let us know, and we can stop. We go at your pace and your comfort level.

All adults were told the following basic script for the 40MWT:

1. Start with both feet on the start line.

2. At start, walk as quickly as possible while maintaining your balance.

3. Walk to the 10-meter mark, turn around, and return to the start mark.

4. You will repeat this four times until you walk 40 meters between the start lines and the 10-meter mark.

5. Go ahead and get ready to start.

6. If you need to stop the trial at any point, please let us know, and we can stop. We go at your pace and your comfort level.

The 5m Walkway Test was used to collect data to compare to the distance covered during the 2-Minute Walk Test. The 40MWT was used to determine how functional an individual's gait was and how the compensations brought on by HOA affected their ability to perform functional movements.

After the data has been collected, the data collected during the 2MWT and 40MWT was determined to be viable and appropriate to use for further analysis. This was integral as we used the two tests to comprehensively understand HOA and

subsequent compensations. Kinematic and kinetic data were recorded and synchronized using Nexus 2.1.1 software (Vicon, Oxford Metrics Ltd., Oxford, UK). Inverse dynamic computation and extraction of hip mechanic variables will be conducted using Visual3D (C-motion, Inc., Germantown, MD, USA). Trajectory and analog data were low-pass filtered at 6 Hz and 25 Hz respectively using a fourth-order Butterworth digital filter based on residual analysis.

Results

Slower gait speeds were associated (β=-24.2, t=-2.27, p=0.04, Figure 1) with greater hip flexion angle in the late stance phase of the involved limb during self-selected walking. Faster gait speeds were associated (β=36.2, t=2.27, p=0.04, Figure 2) with greater hip power output in the early stance phase of the involved limb during self-selected walking. More distance covered during the 2MWT was associated (β=-0.08, t=-2.26, p=0.02, Figure 3) with less hip flexion angle in the late stance phase of the involved limb during self-selected walking. More distance covered during the 2MWT was associated (β=0.11, t=2.06, p=0.05, Figure 4) with greater hip power in the late stance phase of the involved limb during self-selected walking. Slower 40MWT times were associated (β=-53.1, t=-3.74, p=0.00, Figure 5) with greater hip flexion angle in the late stance phase of the involved limb during self-selected walking. These results reflect that hip 5 angle and power were the most affected biomechanical measures of late-stage HOA. The biomechanical measures demonstrate that walking gait adaptations in late-stage HOA include a shorter stride and less muscular power produced per stride. These functional decrements mean that adults with HOA will walk slower and cover less distance, inferring an overall decrease in the ability to perform activities of daily living (ADL).

Table 1. Participant Demographics

Characteristics	Values (n=20)
Age, years	70 (9)
Sex, % male	20 (100.0%)
Mass, lbs.	218.9 (4.5)
Height, cm	178.4 (32.3)
BMI, kg/m2	34.18 (13.24)

Note: All results are reported as mean and standard deviation unless otherwise indicated. BMI, body mass index

Figure 2. Correlation of Gait Speed and Involved Hip Power in Early Stance Phase

p= 0.04, r= 0.47
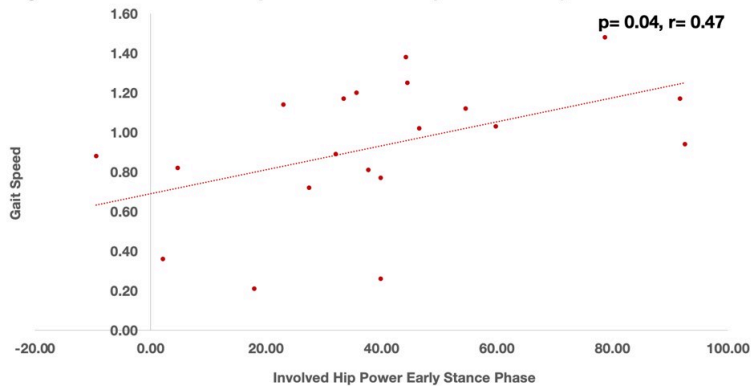
Figure 3. Correlation of Two Minute Walk Test and Involved Hip Angle in Late Stance Phase

p= 0.02, r= -0.53
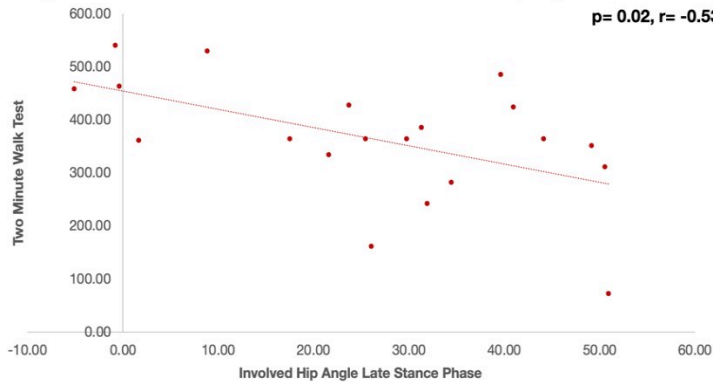
Figure 4. Correlation of Two Minute Walk Test and Involved Hip Power in Late Stance Phase
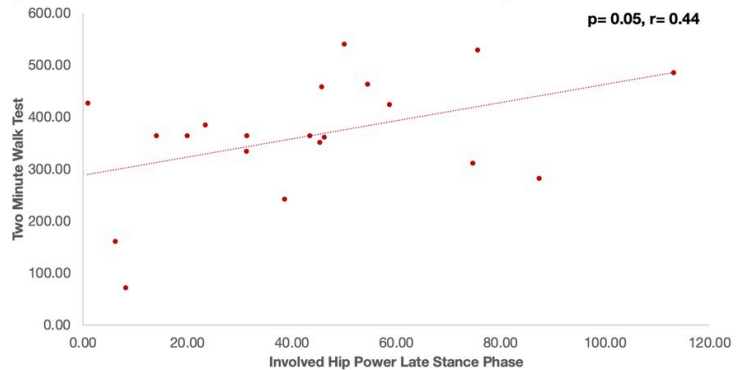
Figure 5. Correlation of Forty Meter Walk Test and Involved Hip Angle in Late Stance Phase
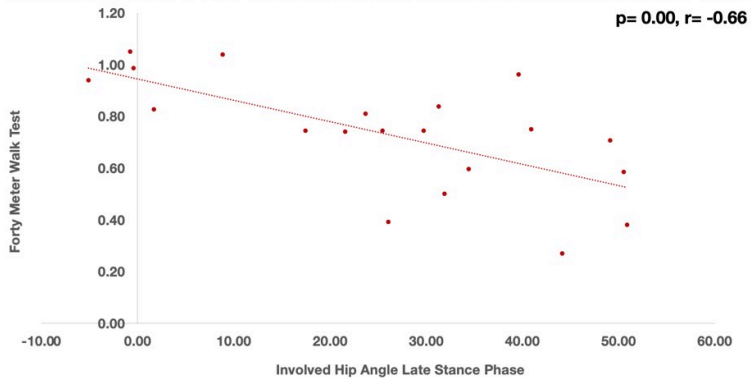
Discussion

The results of this project highlighted deficiencies in two areas, hip angle and hip power, during the functional performance tests of adults with HOA. Hip angle relates to the degrees of flexion and extension between the hip joint and torso, while hip power is the muscular force exerted through the joint. A medium negative correlation (r=-0.47) was found between functional performance in validated gait trials and hip angle. The decreased hip angle in the late stance phase, post-mid-stance until toe-off of the gait cycle, suggests that adults with late-stage HOA take shorter strides. This means a slower walking gait, more steps needed to cover a required distance,

and overall limitations in mobility in ADLs. In contrast, a medium positive correlation (r=0.47) was found between the 2MWT and gait speed. We can conclude that adults with more functional HOA move faster and produce more power per stride. Adults with more functional HOA and the ability to move better regarding ADLs suggest fewer pain symptoms or greater muscular strength. However, further research is needed to explore those relationships further. Limitations in ADLs may also cause a cascade of effects. This includes decreased physical activity, which may lead to chronic diseases offset by regular physical activity. This demonstrates how HOA and the functional adaptations in the gait may lead to drastic overall life changes. Therefore, finding the deficits in hip angle and hip power may lead to the ability of adults to continue to participate in ADLs and not develop additional chronic diseases.

Potential factors influencing the validity of the results include non-diagnosed osteoarthritis or other orthopedic conditions, inaccurate placement of tracking markers, small sample size (n=20), and only male participants being involved in the study. Underlying and non-diagnosed orthopedic conditions can contribute to gait compensation patterns that we concluded to result from HOA. This could mean that HOA could be minorly responsible for the resulting hip angle and power conclusions. Inaccurate placement of tracking markers could also influence the results. While known and consistent anatomical markers were used for tracking marker placement, they were applied by a researcher, which could lead to human error, influencing the conclusions found concerning biomechanical data. The cohort size increased from 10 to 20 over this project. This was done to improve the quality of the relationships, if any were to exist, and reduce bias from

potential outliers skewing the data. Finally, the data may look different if female participants were involved in this study. Different anatomical structures, male versus female, may influence our findings. In addition, female adults may have different compensation patterns than male participants and may influence the resulting hip biomechanics data.

References

Allison, K., et al., Gluteal tendinopathy and hip osteoarthritis: Different pathologies, different hip biomechanics. Gait Posture, 2018. 61: p. 459-465.

Bahl, J.S., et al., Biomechanical changes and recovery of gait function after total hip arthroplasty for osteoarthritis: a systematic review and meta-analysis. Osteoarthritis Cartilage, 2018. 26(7): p. 847-863.

Constantinou, M., et al., Hip joint mechanics during walking in individuals with mild-to-moderate hip osteoarthritis. Gait Posture, 2017. 53: p. 162-167.

Diamond, L.E., et al., Hip joint moments during walking in people with hip osteoarthritis: a systematic review and meta-analysis. Osteoarthritis Cartilage, 2018. 26(11): p. 1415-1424.

Gossec, L., et al., OMERACT/OARSI initiative to define states of severity and indication for joint replacement in hip and knee osteoarthritis. J Rheumatol, 2007. 34(6): p. 1432-5.

Hall, M., et al., Hip biomechanics during stair ascent and descent in people with and without hip osteoarthritis. J Orthop Res, 2017. 35(7): p. 1505-1514.

Harding, P., et al., Do activity levels increase after total hip and knee arthroplasty? Clin Orthop Relat Res, 2014. 472(5): p. 1502-11.

Higgs, J.P., et al., Individuals with mild-to-moderate hip osteoarthritis exhibit altered pelvis and hip kinematics during sit-to-stand. Gait Posture, 2019. 71: p. 267-272.

Hulet, C., et al., [Functional gait adaptations in patients with painful hip]. Rev Chir Orthop Reparatrice Appar Mot, 2000. 86(6): p. 581-9.

Hurwitz, D.E., et al., Gait compensations in patients with osteoarthritis of the hip and their relationship to pain and passive hip motion. J Orthop Res, 1997. 15(4): p. 629-35.

Jones, D.L., A public health perspective on physical activity after total hip or knee arthroplasty for osteoarthritis. Phys Sportsmed, 2011. 39(4): p. 70-9.

Katz, J.N., K.R. Arant, and R.F. Loeser, Diagnosis and Treatment of Hip and Knee Osteoarthritis: A Review. JAMA, 2021. 325(6): p. 568-578.

Kolasinski, S.L., et al., 2019 American College of Rheumatology/Arthritis Foundation Guideline for the Management of Osteoarthritis of the Hand, Hip, and Knee. Arthritis Rheumatol, 2020. 72(2): p. 220-233.

Kolk, S., et al., Gait and gait-related activities of daily living after total hip arthroplasty: a systematic review. Clin Biomech (Bristol, Avon), 2014. 29(6): p. 705-18.

Loureiro, A., et al., Individuals with mild-to-moderate hip osteoarthritis have lower limb muscle strength and volume deficits. BMC Musculoskelet Disord, 2018. 19(1): p. 303.

Loureiro, A., P.M. Mills, and R.S. Barrett, Muscle weakness in hip osteoarthritis: a systematic review. Arthritis Care Res (Hoboken), 2013. 65(3): p. 340-52.

Lunn, D.E., G.J. Chapman, and A.C. Redmond, Hip kinematics and kinetics in total hip replacement patients stratified by age and functional capacity. J Biomech, 2019. 87: p. 19-27.

Malik, A.T., et al., What Are the Costs of Hip Osteoarthritis in the Year Prior to a Total Hip Arthroplasty? J Arthroplasty, 2020. 35(2): p. 313-317.e1.

Maradit Kremers, H., et al., Prevalence of Total Hip and Knee Replacement in the United States. J Bone Joint Surg Am, 2015. 97(17): p. 1386-97.

O'Brien, J., et al., Improving physical activity, pain and function in patients waiting for hip and knee arthroplasty by combining targeted exercise training with behaviour change counselling: study protocol for a randomised controlled trial. Trials, 2018. 19(1): p. 425.

Polkowski, G.G. and J.C. Clohisy, Hip biomechanics. Sports Med Arthrosc Rev, 2010. 18(2): p. 56-62. 7

Rasch, A., et al., Reduced muscle radiological density, cross-sectional area, and strength of major hip and knee muscles in 22 patients with hip osteoarthritis. Acta Orthop, 2007. 78(4): p. 505-10.

Simonsen, E.B., et al., Bone-on-bone forces during loaded and unloaded walking. Acta Anat (Basel), 1995. 152(2): p. 133-42.

Steinhilber, B., et al., Stiffness, pain, and hip muscle strength are factors associated with self-reported physical disability in hip osteoarthritis. J Geriatr Phys Ther, 2014. 37(3): p. 99-105.

Sultan, A.A., et al., Hip Osteoarthritis Patients Demonstrated Marked Dynamic Changes and Variability in Pelvic Tilt, Obliquity, and Rotation: A Comparative, Gait-Analysis Study. Surg Technol Int, 2018. 32: p. 285-292.

van den Bogert, A.J., L. Read, and B.M. Nigg, An analysis of hip joint loading during walking, running, and skiing. Med Sci Sports Exerc, 1999. 31(1): p. 131-42.

Vissers, M.M., et al., Physical functioning four years after total hip and knee arthroplasty. Gait Posture, 2013. 38(2): p. 310-5.

Zeni, J., Jr., et al., Relationship between physical impairments

and movement patterns during gait in patients with end-stage hip osteoarthritis. J Orthop Res, 2015. 33(3): p. 382-9.

Murphy, L.B., et al., Medical Expenditures and Earnings Losses Among US Adults With Arthritis in 2013. Arthritis Care Res (Hoboken), 2018. 70(6): p. 869-876.

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35. Research Reflection by Hunter Blake Carlson

Hunter Blake Carlson

Faculty Mentor: Jesse C. Christensen (Physical Therapy & Athletic Training, University of Utah)

My undergraduate research experience started out as a way for me to build my CV for medical school applications. It evolved into one of the most critical parts of my undergraduate experience and enabled me to closely work with my mentor and start to look at other options besides medical school. I found myself in a lab, applying for grants and scholarships, creating posters, and using my research as my thesis for graduation. This experience has transformed my perception of academia, research, and the health field as a whole. It helped cement my desire to go into the health field and contribute to finding solutions to some of the most pressing issues that

we are facing. I will continue to seek out more opportunities for research, continue to work with my mentor, and continue to work towards my career goal of orthopedic surgery; if anything, this research experience has added even more fuel to the fire.

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36.   **Cognitive Impact of Subjective and Objective Sleepiness**

Joshua Chang and Christopher Depner (Health, Nutrition, and Kinesiology)

Faculty Mentor: Christopher Depner (Health, Kinesiology, and Recreation, University of Utah)

Introduction

1Partial sleep deprivation has been shown to evoke significant cognitive and neurobehavioral deficits amongst adults and adolescents. 2Despite significant research on the correlation between sleep, mood, and cognition, there is a promising avenue for further analysis on understanding potential links

between these variables and the established trait-like interindividual susceptibilities among people with habitual short sleep duration in the real world. 3Findings from a study in postpartum women showed inaccurately interpreting physiological sleep duration could spawn negative emotions and thus worsen cognitive ability throughout the day. Alternatively, such inaccurate interpretations of sleep could also cause the individual to believe they slept well, leading them to overestimate their cognitive abilities after a night of poor sleep. Such circumstances could have deadly public health and safety consequences for individuals in safety critical fields like the medical, military, and transportation industries. The aim of this study is to investigate the links between subjective and objective sleepiness, cognitive performance, and inter-individual vulnerabilities to insufficient sleep.

Methods

12 healthy adults (6M/6F), aged 24±5y (mean±SD), completed a 47-day protocol that consisted of two 4-day laboratory visits separated by 10 days. Prior to each of the two 4-day laboratory visits participants maintained two weeks of ~9 hours/night sleep opportunities. Participants were provided controlled energy balanced diets for two days before and throughout each laboratory visit. Sleep opportunities for each laboratory visit consisted of 9 hours on night 1 (baseline), 5h on nights 2 and 3 (insufficient sleep), and recovery sleep on night 4. We assessed objective sleepiness with the Multiple Sleep Latency Test (MSLT) and subjective sleepiness with the Karolinska Sleepiness Scale (KSS). These tests were administered 5 times every 2h during scheduled wakefulness over the final 24 hours of baseline and during sleep restriction in each of the laboratory visits. Ability to sustain attention was assessed

every 2h during scheduled wakefulness with the Psychomotor Vigilance Test (PVT) during each of the laboratory visits.

Protocol

Figure 1.
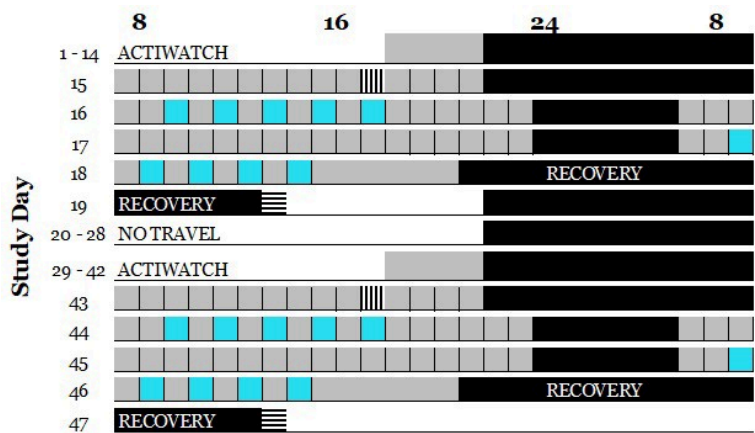
Days 1-14 & 29-42 baseline ambulatory home monitoring with scheduled 9-hour nightly sleep opportunities. Days 15 & 43 laboratory baseline assessments with 9-hour nightly sleep opportunities and energy balanced food intake. Days 16-17 & 44-45 laboratory insufficient sleep assessments with 5-hour nightly sleep opportunities. Sleep was restricted to 5 hours for time in bed by delaying bedtime and advancing waketime by 2 hours each. Vertical bars represent the start of each laboratory visit and horizontal bars represent the end of each laboratory visit. Blue boxes represent MSLT and KSS tests administered in parallel with each other

Results

Figure 2. Increase in objective sleepiness over the course of the study.

* $P < 0.05$ versus baseline
Objective sleepiness (Multiple Sleep Latency Test) during insufficient sleep

MSLT displays increase in objective sleepiness over the course of the study period.

Figure 3.

* $P < 0.05$ versus baseline
Subjective sleepiness (Karolinska Sleepiness Scale) during insufficient sleep

KSS Data shows an increase in subjective sleepiness over the course of the study. Trend parallels that of the objective sleepiness. Subjects felt sleepier when truly objectively sleepier.

Figure 4.

Gray bars represent 5h sleep opportunities. Mixed-model analysis showed a main effect for slower ($P < 0.05$) RT during insufficient versus adequate sleep (baseline). RT, reaction time

Figure 5. Full linear mixed model regression with MSLT, KSS, and study visit with sustained attention (PVT). Analysis shows objective duration is most strongly correlated with PVT performance.

Variable	β Estimate	SEM	P-Value
MSLT	2.141e-05	5.797e-06	0.000222
KSS	-2.345e-05	2.766e-05	0.396542
Study Visit	-2.521e-04	3.296e-05	2.04e-14

Conclusion

As the study day progressed and subjects experienced insufficient sleep, they reported an increasing sense of sleepiness. Objective measures of sleepiness revealed a consistent decline in median PVT reaction time during periods of insufficient sleep in comparison to baseline adequate sleep, and subjects fell asleep faster during these periods. Full mixed model analyses demonstrated that the strongest correlation existed between objective sleepiness and PVT reaction time. These results indicate that subjective sleepiness is an unreliable predictor of sustained

attention, which is a crucial factor for public safety. Therefore, these findings underscore the critical need for remedial action in essential fields such as medicine, military, and transportation, where sleep is often limited.

References

1.   Goel, N., Rao, H., Durmer, J. S., & Dinges, D. F. (2009). Neurocognitive consequences of sleep deprivation. Seminars in neurology, 29(4), 320–339. https://doi.org/10.1055/s-0029-1237117

1.   Banks, S., & Dinges, D. F. (2007). Behavioral and physiological consequences of sleep restriction. Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine,

1.   (5), 519–528. 3Insana, S. P., Stacom, E. E., & Montgomery-Downs, H. E. (2011). Actual and perceived sleep: associations with daytime functioning among postpartum women. Physiology & behavior, 102(2), 234–238. https://doi.org/10.1016/j.physbeh.2010.11.017

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37. **Research Reflection by Joshua Chang**

Joshua Chang

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My undergraduate research experience has been an invaluable pillar of support throughout my college journey. Engaging in research during this phase of my academic life has opened doors of opportunity and allowed me to connect and collaborate with like-minded individuals who have spurred my personal and academic growth. Additionally, it gave me a chance to work alongside experienced professors and researchers in my field of interest, providing me with the tools to explore my passions further. Through my mentor's guidance, I was able to delve deeper into my interests, receive valuable advice, and hone my skills, preparing me for my future goals in medicine and academia. Their support went above and beyond, offering their expertise, knowledge, and compassion. Not only

did they write letters of recommendation for me, but they also served as faculty mentors in multiple projects that I decided to take on. Undoubtedly, my undergraduate research experience has had the most significant impact on my career, serving as a catalyst for my future success in medical school and beyond.

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38. **The Effect of Mild Traumatic Brain Injury on Turning Smoothness**

Cameron B. Jensen; Paula Johnson; Nicholas Kreter; Selena Cho; Leland Dibble (Physical Therapy and Athletic Training); and Peter Fino (Health and Kinesiology)

Faculty Mentor: Peter C. Fino (Health & Kinesiology, University of Utah)

Introduction

Mild traumatic brain injuries (mTBIs; i.e. concussions) can impair a person's motor function, and emerging research

suggests that more dynamic tasks, such as turning, are particularly susceptible to the effects of mTBI [1,2.] While previous work has focused on speed and balance during turning [1,2], the smoothness of a turn may also be important; the ability to perform planned movements in a smooth, non-jerky manner is an important marker of a neurologically healthy motor control system [3]. As the effects of mTBI on the smoothness of turning remain unclear, this study sought to examine the acute and longitudinal effects of mTBI on movement smoothness during turning. We hypothesized that those with an mTBI would exhibit less smooth turns, and smoothness would improve as individuals recovered from their mTBI.

Methods

As part of a larger IRB-approved protocol, 16 healthy controls and 10 mTBI subjects provided informed written consent and walked around a turning course for 150 seconds for each condition. The two conditions were normal and fast walking. The turning course consisted of turns of varying angles. Each subject was tested at an acute time point (within 2 weeks of receiving an mTBI) and then tested again three months later. Kinematic data were recorded from inertial measurement units (IMUs, APDM Opals, Portland, OR), and smoothness of axial rotation was quantified using log dimensionless jerk from the angular velocity data in the transverse (yaw) plane, along with the average lap completion time [4]. Movements with less jerk are described as being smoother. A linear mixed model was fit for the outcome measure of smoothness with fixed effects of lap time, mTBI, time (i.e., visit), the group*time interaction, and random intercepts for each participant.

Figure 1: An IMU was worn on the lumbar region and

collected kinematic data as the participant completed laps around the turning course.
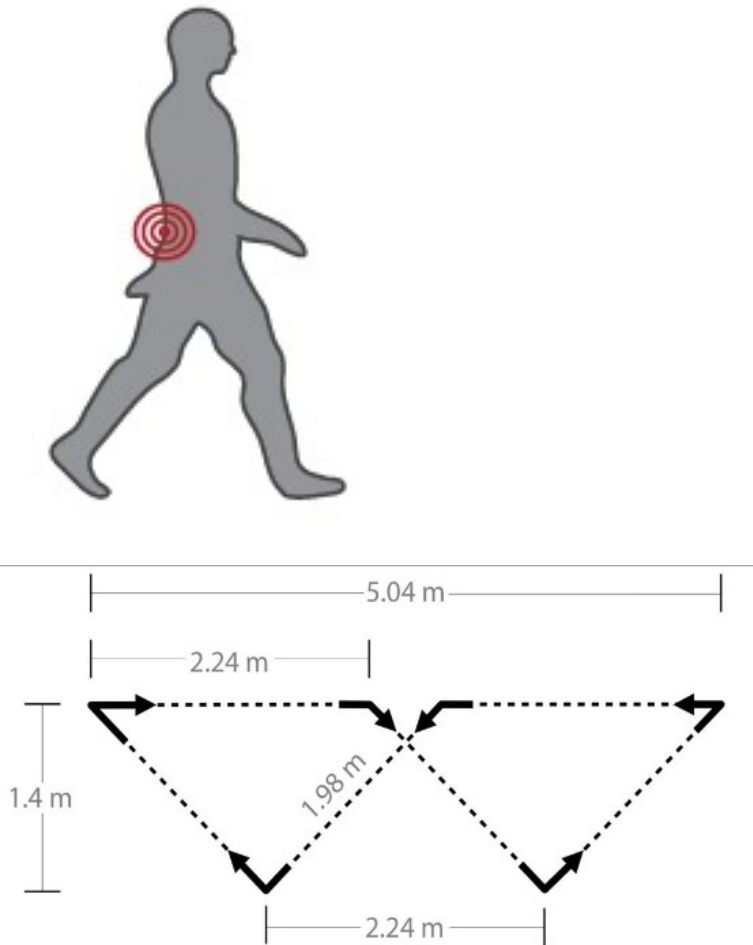
Results and Discussion

A significant group*visit interaction was observed for normal speed walking when adjusting for lap time (p=0.01), where the mTBI group decreased smoothness over time relative to the controls. Faster lap times were associated with smoother axial rotations (p=0.04). A similar result was

observed in the fast-walking condition as a significant decrease in smoothness was observed in the mTBI group (p=0.01), with a noteworthy but non-significant group*visit interaction (p=0.10). These results indicate that faster turning speeds are associated with more smooth turns. While mTBI subjects increased their speed over the course of recovery, their smoothness decreased, which was the opposite of the standard relationship between smoothness and speed /duration[5].

Figure 2: From the initial visit to the follow-up visit the mTBI subjects turned less smoothly while decreasing their lap time (increasing their speed). Smoothness values closer to 0 are considered more smooth.

Fast Walking
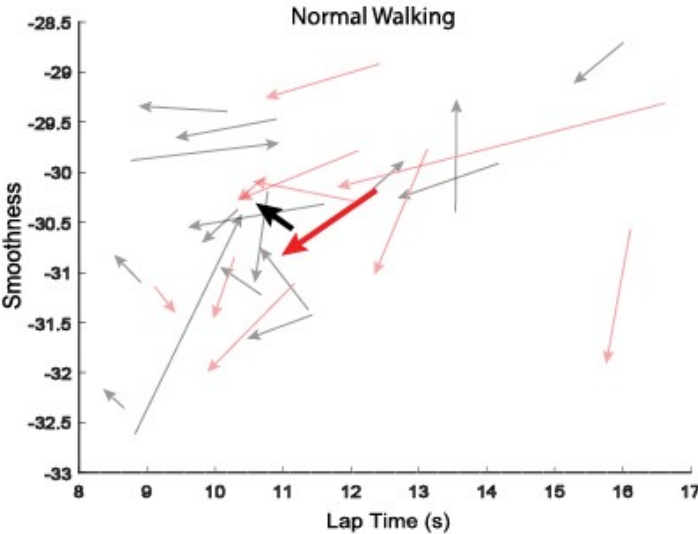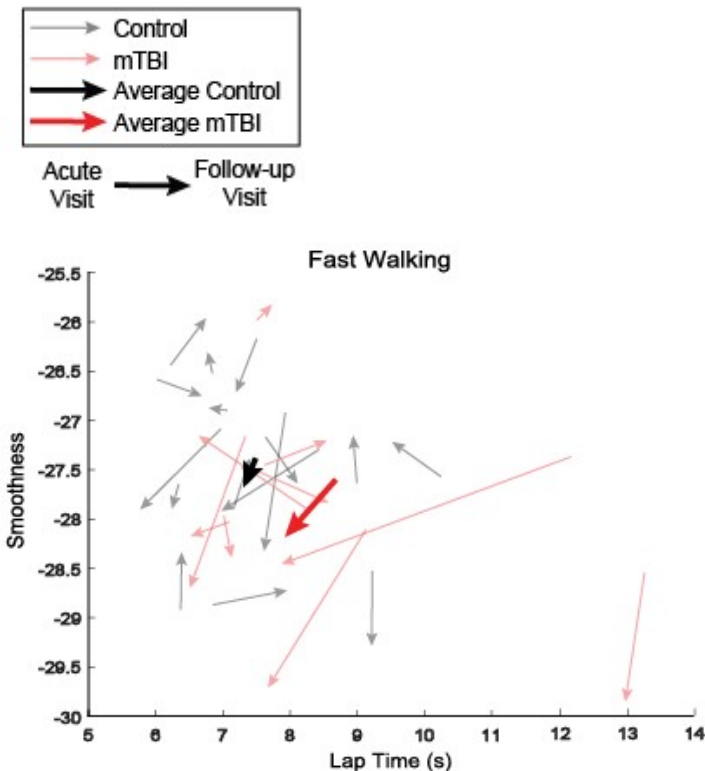
Significance

The decreased smoothness with increasing speed from the initial visit to the follow-up visit suggests that those with an mTBI are experiencing incomplete recovery and may indicate lingering issues that are not captured by turning speed alone. This causes concern for symptom provocation and injury risk. Future research will look at other factors that may impact smoothness (e.g. symptoms), and explore clinically meaningful changes in smoothness.

Acknowledgments

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References

[1] Fino et al., (2018). *J Neurotrauma* 35(10): 1167-1177.

[2] Fino et al., (2016). *J NeuroEngineering Rehabil*, 13, 65.

[3] Hogan et al., (2009). *J of Motor Behavior*, 41:6, 529-534.

[4] Parrington et al., (2021). *Gait & Posture*, 90, 245-251.

[5] Balasubramanian et al., (2015). *J NeuroEngineering Rehabil*, 12, 112.

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39. Development of a Novel Fluorogenic-Based Assay to Measure Chaperone Mediated Autophagy in Cells and Tissues

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Faculty Mentor: Rajeshwary Ghosh (Nutrition & Integrative Physiology, University of Utah)

Absract

Misfolded and damaged proteins are usually checked by protein degradation pathways which are essential to maintain proteostasis. Chaperone-mediated autophagy (CMA) is a protein degradation pathway unique to mammalian cells. CMA uses protein type 2a (LAMP2A) receptors, which are specific to CMA, to target proteins that contain a KFERQ-like motif for lysosomal degradation. LAMP2A protein expression has been used previously to assess CMA function. In this project, we have established a fluorescent-based CMA activity assay to measure CMA in cells and tissues. We first extract intact lysosomes from adult mouse heart, liver, and kidney and cultured mouse embryonic fibroblasts. We then aim to demonstrate the use of KFERQ-AMC (Lys- Phe-Asp-Arg-Gln-AMC) fluorogenic substrate to measure CMA activity. AMC when attached to the substrate remains in the quenched state and does not fluoresce. The cleavage of KFERQ-AMC due to lysosomal hydrolysis causes free AMC to release which is excited at 355 nm and fluoresces at 460 nm. By measuring the cleavage, we can measure the levels of CMA activity. To determine the flux, we used another set of samples treated with E64D, (L-trans-Epoxy-succinyl-leucylamido(4-guanidino)butane), a cathepsin inhibitor, which would serve as controls. Until now, we found that mouse liver shows the highest CMA activity (6-fold) followed by kidney (2.4-fold) and heart (0.3-fold) at 5 hours. The levels of KFERQ-AMC degradation in the different tissues positively correlated to the LAMP2A protein expression. We also found that KFERQ motif is specific for CMA degradation, as the negative control

peptides showed no fluorescence. Further KFERQ-AMC cleavage was blocked by E64D treatment, which allowed us to measure CMA flux. We believe that the CMA activity assay would allow us to dynamically monitor CMA flux in a host of cells and tissues and help to identify and validate various CMA activators as potential drugs for the prevention or treatment of different pathologies.

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40. Dietary Blueberries to Improve Oral Dysbiosis

Nizhoni Dawn Porter and Anandh Babu Pon Velayuthum (Nutrition and Integrative Physiology)

Faculty Mentor: Anandh Babu Pon Velayuthum (Nutrition and Integrative Physiology, University of Utah)

The gut and oral cavity are the two most significant microbial habitats and are closely connected through the digestive pathway. Evidence indicates microbial transmission (oral-to-oral and gut-to-oral) regulates the pathogenesis of diseases such as cancer and is mediated through the oral-to-gut microbiome axis. An imbalance in the oral microbes (oral dysbiosis) is implicated in oral diseases and systemic diseases such as inflammatory bowel disease, cardiovascular disease,

and Alzheimer's disease. Our lab recently showed that dietary supplementation of blueberries improves diabetes-induced gut dysbiosis. Dietary blueberries increased the abundance of commensal microbes and decreased the abundance of opportunistic microbes in diabetic mice. Blueberries contain phytochemicals such as anthocyanins which act as prebiotics and promote the growth of beneficial gut microbes. In our current study, we are evaluating whether supplementation of blueberry improves a high-fat diet and antibiotics-induced oral dysbiosis in a preclinical model. Mice were divided into four groups: control (C), induced oral dysbiosis in a high-fat diet fed and treated with antibiotics (HFA), and HFA supplemented with a diet containing a nutritional dosage of freeze-dried wild blueberries (HBA). The Antibiotics cocktail was supplemented to the mice in the drinking water. We isolated the bacterial DNA from oral and cecum samples. Currently, we are analyzing oral and gut microbiomes using 16s rRNA amplification. We expect that the probiotic effect of anthocyanins and the translocation of bacteria (between the oral and gut microbiota) could help to replenish the gut and oral microbiome once destroyed and/or altered. Our study will identify whether dietary blueberries improve oral dysbiosis by modulating the oral-gut microbiome axis.

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41. **Research Reflection by Nizhoni Dawn Porter**

Nizhoni Dawn Porter

Faculty Mentor: Anandh Babu Pon Velayuthum (Nutrition and Integrative Physiology, University of Utah)

I am a half-Navajo student at the University of Utah as a Predental Health Society and Policy major with a minor in History. I hope to continue in dental school and learn more about what goes into dental research and explore that side of the dental industry. My goal is to help the Native American community through my clinical work and potentially through research. This opportunity has given me an edge in my application to dental school because it shows my research interest as well as allows me to see how research labs function, what goes into creating a research project, how to apply for research funding, learned to problem solve in a lab setting,

how to work in a research lab, and has given me hands-on experience working in an animal research lab. This experience has helped me better understand being a researcher and given me the ability to understand other studies and papers to stay up to date on the most current research and give my patients the best care possible. This knowledge has helped me on my journey to providing quality care to others in the Native American community and, hopefully, in inspiring other Native students to continue their journey in education.

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42. **A Feasibility Trial to Determine the Effect of Mindfulness on Weight-Loss Maintenance**

Jaclynn K. Smith; Selene Tobin; Marc-Andre Cornier; and Tanya Halliday (Health, Kinesiology, and Recreation)

Faculty Mentor: Tanya Halliday (Health and Kinesiology, University of Utah)

PURPOSE

Over 70% of all adults in the U.S. have overweight and

obesity. Weight loss is often recommended to improve overall health and risk of disease. However, weight regain following intentional weight loss is common and diminishes initial improvements. Therefore, interventions aimed at attenuating weight regain following weight loss are of crucial importance. Mindfulness-based interventions are a promising and novel approach in mitigating weight re-gain. Therefore, the aim of this study was to investigate the effects of a mindfulness intervention to prevent weight regain in weight-reduced adults. METHODS: Women (age 40.2 ± 10.8; BMI: 31.6± 5.2) who recently achieved a 7% reduction in body mass within the past 2 months were enrolled into an 8-week KORU mindfulness-mediation, plus daily meditation homework. At baseline and post-intervention body mass, dietary restraint, disinhibition, and hedonic hunger (via the Three-Factor Eating Inventory); and physical activity (via IPAQ) were evaluated. To compare change in body mass to a standard weight loss maintenance intervention (Diabetes Prevention Program [DPP]Post Core Curriculum) 8-week weight loss data from historic controls matched on age, sex, and BMI (n=5) was utilized. RESULTS: Weight loss was maintained following the 8-week KORU mindfulness group from pre- to post-intervention for dietary restraint (pre: 11.7 ± 4.1; post: 12.7±4.5; p=0.27), disinhibition (pre: 6.3 ± 4.4; post:6.6 ± 4.8; p=0.69), or hedonic hunger (pre: 3.7 ±2.6; 2.9 ± 3.8; p=0.29). Additionally moderate-to-vigorous physical activity (pre: 121141.1 ± 8706.3 METmins/week; post: 7422 ± 5842.8 METmins.week; p=0.17) and sedentary time (pre: 4274.3 ± 1544.9 min/week; post: 2655.7 ± 1508.8 min/week; p=0.43) were also not changed following the KORU intervention. CONCLUSIONS: Mindfulness-interventions are feasible and an efficacious approach to short-

term weight loss-maintenance. Future trials that include matched controls and longer term follow up are needed.

INTRODUCTION

- Weight-loss maintenance following significant weight loss is an often-neglected period of weight loss interventions. Frequently, within two years of weight loss, nearly half of weight is regained, and up to 80% of weight is regained within five years1.

- Mindfulness meditation is a formal practice of mental training that can lead to the use of mindfulness in informal settings2.

- Mindfulness has shown positive results as a complement to traditional medical and psychological treatments2,3, and is therefore an intervention of interest for the weight-maintenance phase.

METHODS

PARTICIPANTS: Females (n=7) who recently experienced significant weight loss (age: 40.3 ± 3.8 years; BMI before weight loss (age: 31.6 ± 1.9 kg/m2; BMI after weight loss: 27.5 ± 1.5 kg/m2) participated in an 8-week KORU mindfulness intervention.

STATISTICAL ANALYSIS: Paired t-tests were used to compare pre-post data. SPSS was used for all analysis. Alpha was set at <0.05. All data is shown as average ± standard error mean. Graphpad PRISM was used to display results.

Baseline Measurements: We evaluated Body composition via DEXA Three Factor Eating Questionnaire (Figure 1).

Post-Intervention Measurements: Body composition via DEXA Three Factor Eating Questionnaire (Figure 1).

The KORU Intervention as well as a Control intervention was used to evaluate weight-loss maintenance while being given a mindfulness curriculum (Figure 1).

Figure 1. Study Timeline for the intervention and control groups. TFEQ: Three Factor Eating Questionnaire; IPAQ: Internation Physical Activity Questionnaire; DPP: Diabetes Prevention program.
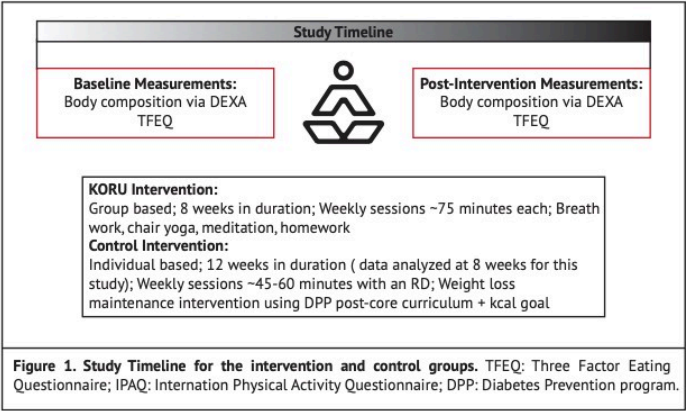
RESULTS

- There were no differences observed in pre- to post-KORU intervention in body fat percentage (p>0.05; Figure 2), nor in any domains evaluated by the TEQ (p>0.05; Figures 3-5).

Figure 2: Body Fat Percentage (KORU intervention)
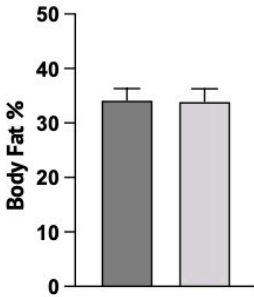
Figure 3: TFEQ Dietary Restraint
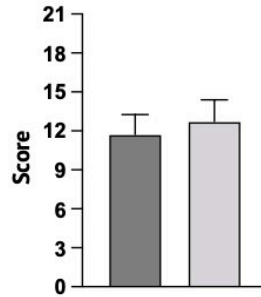
Figure 4: TFEQ Hedonic Hunger
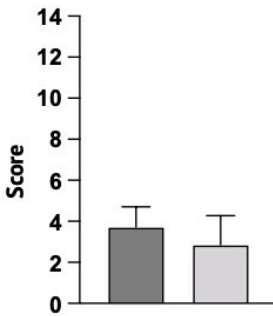
Figure 5: TFEQ Dietary Disinhibition
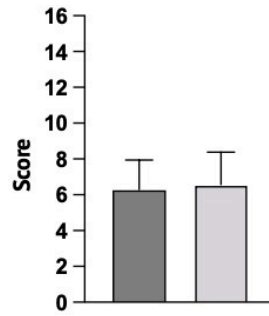
Baseline   Post-Intervention

- There were no differences in weight change observed when comparing the KORU intervention to the historic DPP control intervention (p>0.05; Figure 6).

• There were no differences observed pre- to post-KORU intervention in body fat percentage (p>0.05; Figure 2), nor in any domains evaluated by the TFEQ (p>0.05; Figures 3-5).

• There were no differences in weight change were observed when comparing the KORU intervention to the historic DPP control intervention (p>0.05; Figure 6).

Figure 6: KORU vs. DPP Weight Change
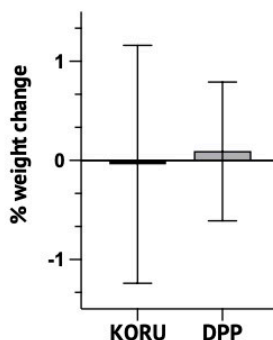
CONCLUSIONS

- Body Composition did not change following the KORU mindfulness intervention, and body weight was not statistically different than the DPP control group at 8-weeks.

- Dietary restraint, disinhibition, and hunger did not change following the intervention.

- These data suggest mindfulness to be am intervention of interest during the weight maintenance period to maintain clinically significant weight loss.

REFERENCES

1. Hall KD, Kahan S. Maintenance of Lost Weight and Long-Term Management of Obesity. Med Clin North Am. 2018;102(1):183-197.doi:10.1016/j.mcna.2017.08.012

2. Grossman P, Niemann L, Schmidt S, Walach H. Mindfulness-based stress reduction and health benefits: A meta-analysis. Journal of Psychosomatic

Research. 2004;57(1):35-43.

3. Wielgosz J, Goldberg SB, Kral TRA, Dunne JD, Davidson RJ. Mindfulness Meditation and Psychopathology. Annu Rev Clin Psychol. 2019;15:285-316.doi:10.1146/annurev-clinpsy-021815-093423

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43. **Research Reflection by Jaclynn K. Smith**

Jaclynn K. Smith

Faculty Mentor: Tanya Halliday (Health and Kinesiology, University of Utah)

My undergraduate research experience has been life changing. The vast research opportunities that the University of Utah has was the deciding factor for me to attend, however I did not know how much it would truly benefit me. Not only do I have real research lab and procedure experience, but I also gained incredible mentors, patient interaction, and the ability to be comfortable reading and interpreting published research. My future education goal is to apply and attend medical school so that I can pursue an MD and practice medicine. Research has helped me immensely in the pursuit of this goal because it is both a requirement and a necessity for practicing physicians to be involved in and comfortable reading and interpreting new research. Medicine is changing all the time and research is at

the forefront of this change. I am incredibly grateful for the impact my undergraduate research experience has had on me.

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44. "Out of Duty and Love": The Similar Experiences and Needs of Diverse Family Caregivers

Emi Wickens; Anne V. Kirby (Occupational and Recreation Therapies); Sarah A. Neller (Nursing); Megan Hebdon (Health, University of Utah); Debra L Scammon (Marketing); Rebecca L. Utz (Sociology); Kara B. Dassel (Nursing); Alexandra L. Terrill (Occupational & Recreational Therapies);

and Lee Ellington
(Nursing)

Faculty Mentor: Anne V. Kirby (Occupational & Recreational Therapies, University of Utah)

Objective: This is a qualitative research study that aims to understand the similarities across a variety of diverse caregiving experiences, including a broad range of age, caregiving relationship, and chronic illness or disability.

Background: A family caregiver, also referred to as an informal caregiver, aids with an array of tasks for an individual with a chronic health condition or disability who cannot safely function on their own. Previous research has focused on specific caregiving situations, leading to much uncertainty concerning the experiences and needs of caregivers on a broad spectrum. This study aims to discover the shared life of diverse caregivers to help effect change in current and future policies to better support a broad population of caregivers.

Method: Three virtual focus groups were held with a purposive sample of caregivers (n=26) caring for an individual with a chronic disability or health condition that they have an established relationship with. We used an inductive, iterative process to guide our thematic analysis.

Results: The participants largely demonstrated shared experiences and needs among their diverse caregiving situations. We identified common themes of experience to be: Doing it All; Changes Over Time; Complex Emotions; and Expectations. We identified common themes of needs to be:

Breaks and Care of Self; Help, Support, and Resources; and Understanding and Recognition.

Conclusion: Our findings suggest the shared experiences and needs among caregivers are significant, despite a myriad of caregiving situations. The deeper understanding of life as a caregiver obtained from this study is applicable to future research, medical professionals, and new and existing policies concerning caregiving.

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45. Research Reflection by Emi Wickens

Emi Wickens

Faculty Mentor: Anne V. Kirby (Occupational & Recreational Therapies, University of Utah)

My undergraduate research gave me the opportunity to apply what I had been learning in school to a real life problem that I am passionate about. Before I began this project, I was very intimidated by research, but now I feel like I have the skills to perform inquiry based investigations independently. I am applying to medical school and plan to become a physician, so this work is very important to my future goals as it provides me with a unique perspective on healthcare. As a public health major, I was very excited to create a policy recommendation based on my own project.

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46. Intramuscular Macrophage Delivery Improves Aged Muscle Function After Disuse Atrophy

Elena Yee and Patrick Ferrara

Faculty Mentor: Micah Drummond (Physical Therapy & Athletic Training, University of Utah)

Abstract

Aging muscle and extended periods of disuse and/or injury leading to muscle atrophy compromises the immune system causing a dysfunctional macrophage response in muscle regrowth. This study examines the effects of injecting young adult and aged recipient mice with pro-inflammatory

macrophages into the hindlimb muscles to detect if it stimulates an immune response that improves muscle recovery, after following a period of disuse atrophy. The experiment involved the recipient mice that underwent 2-weeks of hindlimb unloading muscle atrophy followed by a 4-day recovery period. Bone marrow-derived macrophages were isolated from three donor groups: young adult mice, aged mice, and 4-day reloaded aged mice. These cells were cultured in a dish, changed to pro-inflammatory macrophages, and then injected into the right hindlimb of the recipient mice after 1-day of ambulatory recovery from hindlimb unloading. On day 4 of recovery, the mice were humanly euthanized for analysis of the soleus muscle. Our results indicate that the delivery of pro-inflammatory macrophages from the three donor groups did not affect muscle mass when compared to the vehicle (Phosphate buffer saline, PBS) in young adult and aged mice; however, the delivery of the pro-inflammatory macrophages improves/rescues aged mice muscle recovery following disuse.

Introduction

Extended periods of muscle disuse and/or injury can lead to muscle atrophy within the skeletal muscles (1). Macrophages found within our immune system regulates/defends against infection/injury via processes of inflammation and phagocytosis by releasing growth factors that play a critical role in muscle regeneration and organization (2). The crosstalk between the immune system and skeletal muscle is known to be dysfunctional with aging during the recovery from disuse atrophy (3). This is concerning especially regarding the elderly population since they are more prone to increased risk of falling, developing an illness, and worsening disease

complications (4). These macrophages have been identified as a key immune cell responsible for this dysfunctional crosstalk with aging (5).

The delivery of pro-inflammatory macrophages into the hindlimb muscles of young and aged mice has not been examined before. Therefore, to investigate this specific immunotherapy treatment, we induced muscle atrophy via hindlimb unloading for 14 days and administered pro-inflammatory macrophages from three different donor groups: young adult mice, aged mice, and 4-day reloaded aged mice into the hindlimbs of both young adult and aged mice 1 day following hindlimb unloading recovery. The purpose of this investigation is to determine if the delivery of these pro-inflammatory macrophages improves muscle recovery in aged mice. By performing this experiment, it could potentially lead to new treatments and exciting immunotherapies that could help skeletal muscle recovery following disuse atrophy especially regarding the elderly population.

Methods

Experimental outline

Both the young adult (3-6 months of age) and aged (23-26 months of age) C57BL/6 strain mice were divided into two experimental groups: the donor and recipient. Bone marrow-derived macrophages (BMDMs) were obtained from the three following donor groups which were used for intramuscular injections: ambulatory young adult mice, ambulatory aged mice, and aged mice that were hindlimb unloaded and reloaded for 4 days. The recipient young adult and aged mice underwent

2 weeks of hindlimb unloading followed by 4 days of reloading. One day after the HU intervention (Day 15), intramuscular injections were performed. Pro-inflammatory macrophages from the donor were delivered into the right hindlimb of young adult and aged recipient mice, while phosphate buffer saline (PBS, Vehicle) was administered into the left hindlimb. The mice continued to be ambulatory for three more days and then were euthanized. The soleus muscle was obtained for isometric force production and the muscle mass was recorded for analysis.

Bone-Marrow Derived Macrophage Isolation, Polarization, Delivery

Bone marrow-derived macrophages (BMDMs) were isolated from the three following donor groups: ambulatory young adult mice, ambulatory aged mice, and aged mice that were hindlimb unloaded and reloaded for 4 days. The mice were euthanized to obtain the femur and tibia bones where they were dissected and cleaned. A gauge needle with sterile PBS was used to flush the bones which were centrifuged and then resuspended in red blood cell lysis buffer. The cells were centrifuged again and resuspended in D10 media with macrophage-colony stimulating factor (M-CSF). These cells were plated for 4 days and were polarized on day 7 with D10 media, M-CSF, lipopolysaccharide, and interferon γ for 24 hours. After 24 hours, the cells were washed with 2x with PBS and scraped in trypsin. Cells were centrifuged and resuspended in PBS (2 million cells/100μL) that were rear-loaded into a syringe. 100μL of the polarized pro-inflammatory macrophages were administered into the right hindlimb of the recipient mice.

Ex-vivo Muscle Force Production

The soleus muscles were carefully sutured at each tendon and dissected from the mice while under isoflurane anesthesia before the measurement of muscle function. Force-frequency analyses were performed via stimulations ranging from 10-200 Hz with 1 minute between each stimulation. A dual-mode lever force transducer software (Aurora Scientific) was used to measure force and analyze data. The soleus muscle mass and length were measured and recorded.
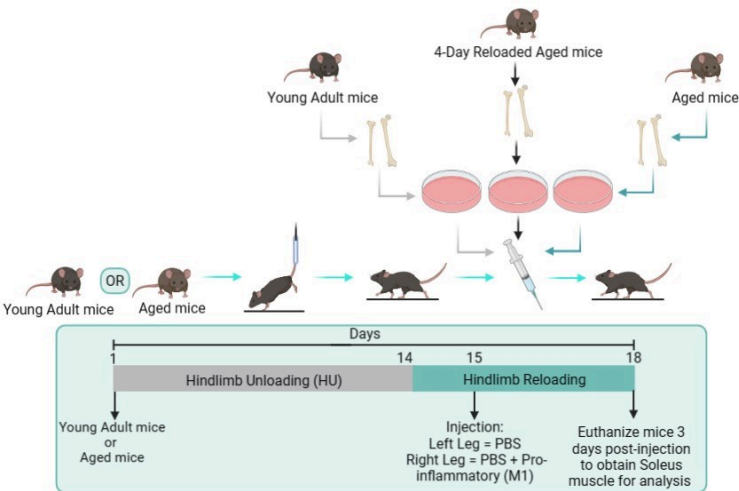
Figure 1: Schematic of experimental design.

Results

Our results indicate that the delivery from any of the three donor groups actually helped improve muscle recovery in aged mice that underwent the hindlimb unloading intervention. As indicated in figures 2-4, part D, there was an effect of treatment and the asterisk indicate that within that specific time point on the force-frequency curve, there was a significant difference between the vehicle and the aged pro-inflammatory macrophages. While, on the other hand, the young adult mice that underwent the hindlimb unloading intervention had no

effect of treatment and no significant difference within the force-frequency curves (figures 2-4, part B). The bar graphs found in figures 2-4, part A and C, indicate that the muscle mass of the soleus for both the vehicle and the macrophage injections had no significant difference.
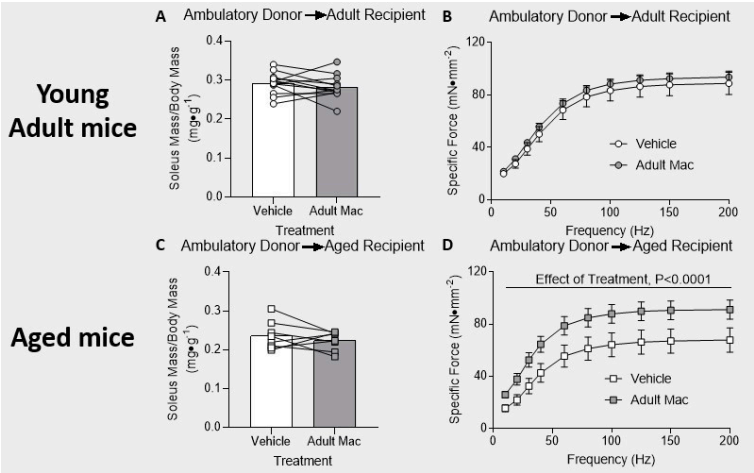
Figure 2: Delivery of young adult pro-inflammatory macrophages improved recovery in aged mice after hindlimb unloading. (A & B) Young adult mice and (C & D) aged mice were injected with PBS in the left hindlimb and young adult pro-inflammatory macrophages in the right hindlimb. (A & C) Mass of the soleus muscle relative to the body mass of the mice. P-values are based on a two-tailed t-test. (B & D) Tetanic force production during a force frequency curve. A 2-way ANOVA with sidak's multiple comparisons test was used. Data are presented as mean ± SEM.
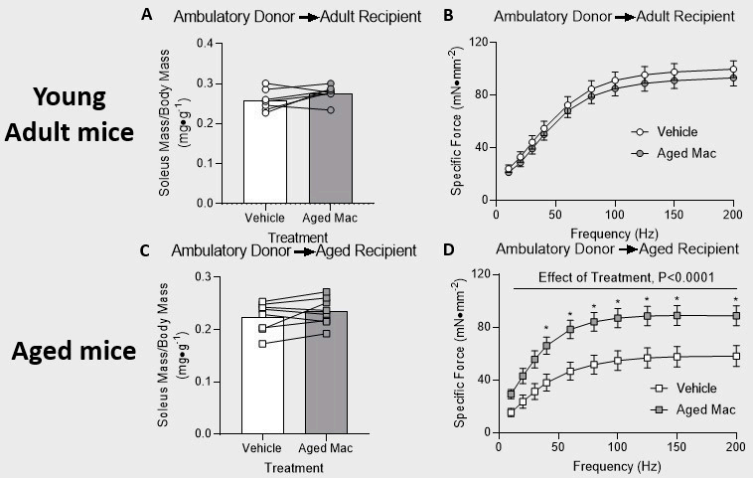
Figure 3: Delivery of aged pro-inflammatory macrophages improved recovery in aged mice after hindlimb unloading. (A & B) Young adult mice and (C & D) aged mice were injected with PBS in the left hindlimb and aged pro-inflammatory macrophages in the right hindlimb. (A & C) Mass of the soleus muscle relative to the body mass of the mice. P-values are based on a two-tailed t-test. (B & D) Tetanic force production during a force frequency curve. A 2-way ANOVA with sidak's multiple comparisons test was used. Data are presented as mean ± SEM. *P<0.05 versus PBS.
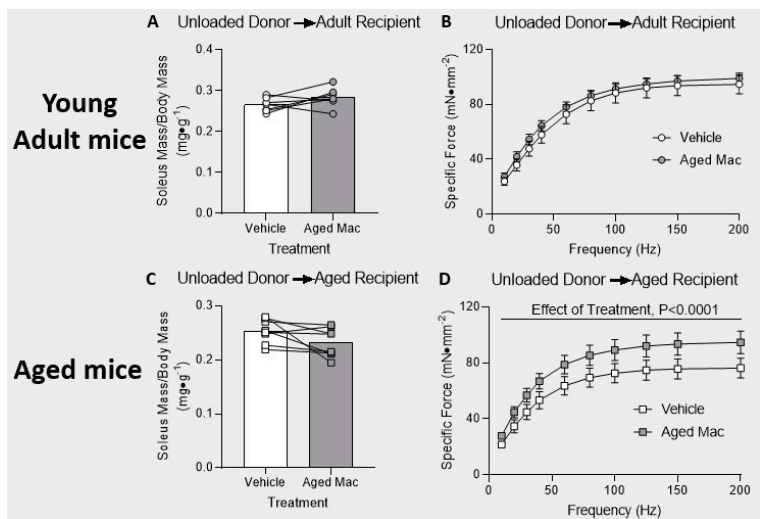
Figure 4: Delivery of pro-inflammatory macrophages from 4-day reloaded aged mice improved recovery in aged mice after hindlimb unloading. (A & B) Young adult mice and (C & D) aged mice were injected with PBS in the left hindlimb, and aged mice reloaded for 4 days following 14 days of HU (unloaded) pro-inflammatory macrophages in the right hindlimb. (A & C) Mass of the soleus muscle relative to the body mass of the mice. P-values are based on a two-tailed t-test. (B & D) Tetanic force production during a force frequency curve. A 2-way ANOVA with sidak's multiple comparisons test was used. Data are presented as mean ± SEM.

Discussion/Conclusion

In summary, the delivery of pro-inflammatory macrophages via intramuscular injection from young adult mice, ambulatory aged mice, and aged mice reloaded for 4 days following 14 days of hindlimb unloading helps rescue aged mice muscle recovery following disuse atrophy. Therefore, the delivery of macrophages to the soleus muscle improves muscle recovery in aged mice. These findings allow for new and exciting

immunotherapies to be implemented in future studies. Two potential follow-up studies could consist of understanding/ determining the mechanisms that helped improve the delivery of pro-inflammatory macrophages to aged mice and if the delivery of other macrophages, other than pro-inflammatory, can also help improve muscle recovery in aged mice. These further investigations would help to solidify our findings and understanding of the role of macrophages to help aid in muscle recovery after disuse atrophy. Page Break

References

1. Nunes, Everson A et al. "Disuse-induced skeletal muscle atrophy in disease and nondisease states in humans: mechanisms, prevention, and recovery strategies." American journal of physiology. Cell physiology vol. 322,6 (2022): C1068-C1084.

1. Hammers, David W et al. "Anti-inflammatory macrophages improve skeletal muscle recovery from ischemia-reperfusion." Journal of applied physiology (Bethesda, Md. : 1985) vol.118,8 (2015).

1. Wang, Ying et al. "Aging of the immune system causes reductions in muscle stem cell populations, promotes their shift to a fibrogenic phenotype, and modulates sarcopenia." FASEB journal: official publication of the Federation of American Societies for Experimental Biology vol. 33,1 (2019): 1415-1427.

1. Fried, L P et al. "Frailty in older adults: evidence for a phenotype." The journals of gerontology. Series A, Biological sciences and medical sciences vol. 56,3 (2001): M146-56.

1. Fix, Dennis K et al. "Disrupted macrophage metabolic reprogramming in aged soleus muscle during early recovery following disuse atrophy." Aging cell vol. 20,9 (2021): e13448.

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47. Research Reflection Elena Yee

Elena Yee

Faculty Mentor: Micah Drummond (Physical Therapy & Athletic Training, University of Utah)

My undergraduate research experience has been amazing. I am very grateful that I was able to actively participate in various aspects/areas of the research efforts alongside Dr. Drummond and his lab members. They have been very supportive throughout my experience in the lab and have helped me grow as an individual. I was very glad that I had the opportunity to work with Patrick Ferrara, a postdoctoral fellow in the Drummond lab, to conduct this particular project/experiment. Through this project, we were able to implement immunotherapies that helped rescue aged mice muscle recovery after following disuse atrophy. This was a very fascinating finding because this could be a potential treatment that can help the elderly population after they have

undergone a period of disuse.

I have learned a lot from Dr. Drummond and his lab members. They all will have a big impact towards my educational and future goals. I am wanting to pursue a career in the area of Regenerative Medicine in the future. The laboratory techniques and data analysis that I have been taught in the Drummond lab will be very translatable for this particular field of research that I am interested in. And this project has been great to help me towards fulfilling my career aspirations. Regenerative laboratory research and medicine will potentially lead to new advancements and alternative medical treatments/therapies for patients, given that the human body already has some natural ability to heal itself. Through working in Regenerative Medicine, in the future, I hope to bring/develop new methods; so, people/patients can live healthy and active lives.

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SECTION VII

College of Humanities

48. **Discord between transgender women and TERFs in South Korea**

Sooyoun Bae

Faculty Mentor: Scott Morris (Writing & Rhetoric, Asia Campus)

Abstract

This paper introduces the conflict between transgender women and trans-exclusionary radical feminists (TERFs) in South Korea. Specifically, researchers state that the main reason TERFs exclude transgender people is the threat of anti-feminists these days, and this study will examine the discord between feminists and anti-feminists as well. In other words, the discourse of exclusion is created in order to protect themselves from attacks and threats. In addition, TERFs claim

that trans women and feminists are incompatible since they believe trans women reinforce gender stereotypes. However, transgender people are suffering due to prevalent personal and systematic discrimination in society, and this paper suggests some specific incidents of the discrimination they are undergoing. Furthermore, it discusses the female-only spaces and the fear of men's invasion, which leads to excluding trans women from these spaces.

Introduction

In South Korea, conflicts between transgender people and a group called TERFs are ongoing. TERFs often exclude trans women from the category of women, despite their female gender identity, and claim that trans women are against their ultimate goal: the dissolution of gender and patriarchy. As a result, transgender people are also alienated and experience discrimination against them. This paper will examine the conflict between TERFs and transgender women, focusing on the claims, hardships, and the meaning of women-only spaces to glance at both groups' stances and further consider a more mature society.

The United States National Center for Transgender Equality (2016) defines transgender as "a broad term that can be used to describe people whose gender identity is different from the gender they were thought to be when they were born." On the other hand, Miller and Yasharoff (2020) explain trans-exclusionary radical feminists (TERFs) as a group of people who extremely prioritize women's rights and resolutely oppose misogyny.

Stances of TERFs

The major reason TERFs exclude transgender people is that they deny the existence of gender identity itself. A cisgender woman named Maya Forstater posted that people cannot change their biological sex on her Twitter (Bowcott, 2019), saying, "There are two sexes. Men are male. Women are female. It is impossible to change sex. These were until very recently understood as basic facts of life" (Sullivan & Snowdon, 2019). Eventually, she was accused of using "offensive and exclusionary" language and opposing government proposals to accept people's self-identity as the opposite sex" (Bowcott, 2019). J.K. Rowling, the author of the famous novel Harry Potter series, advocated Forstater and posted that it was unfair and extreme to fire her from the job for stating sex is real, and she was criticized as well for excluding transgender people (Sullivan & Snowdon, 2019).

Shared Experience of Misogyny

Following the instance mentioned above, TERFs in South Korea also highlight severe misogyny and emphasize protecting women's rights. H. Lee (2019) analyzed that the background of TERFs is common experiences regarding sexual assaults, misogyny, and hate crimes, specifically after the Gangnam station murder case. A 34-year-old man murdered a woman in her twenties in a public restroom near Gangnam station, which is one of the most crowded

areas in Seoul, on May 17, 2016 (Online News Team, 2016). The case has been controversy about whether it is a hate crime reflecting misogyny as the assailant said during the police investigation that he committed the crime because he felt like the victim had ignored him as other women did to him, even though they did not know each other personally (Online News Team, 2016). H. Lee (2019) found that this case triggered a lot of Korean women to recognize feminism and become feminists, and the unrest of women increased as the murder happened in a women's restroom, which implies women cannot be safe even in female-only spaces. On May 17, 2021, some civic organizations participated in the struggle for the fifth anniversary of the Gangnam station murder and to win natural rights such as "the right not to be scared at night or anywhere, the right not to have anyone try to break into the house without permission, the right not to experience digital sex crimes, dating violence, and sexual violence forced by power" (News1, 2021). The researcher named Seunghwa Jeong (2018) analyzed that the 'biological female' category was initially constructed to defend from anti-feminists who use transgender rights to attack feminism rather than in order to exclude transgender women. Ri-Na Kim (2017), a researcher at the Korean Women's Development Institute, examined the two most representative websites of radical feminists. In the article, she states that transgender women

were naturally perceived as subjects of solidarity initially; however, transgender women and the category of women started to become controversial in August 2016 while preparing an online community for female sexual minorities. R-N. Kim (2017) also analyzes that transgender women's femininity is regarded as exaggerated and wrong, and they have been blamed by radical feminists who claim that transgender women cause confusion on the category of women and "re/produce" existing femininity (p. 126).

Moreover, there are more severe and violent anti-feminism these days. A feminist organization named "Haeil," which means tsunami in Korean, was attacked by an anti-feminist organization named "New Men's Solidarity" on August 22, 2021, during the protest against the politicization of anti-feminism (Chaigne, 2021). The anti-feminists rushed into the protest, shouted assaulting words, threatened them, surrounded them with water guns, and said:

> Look at all of these feminazis! That's right, run away! At least you'll get a bit of exercise! ... So you got water on you? Are you angry? God, there are so many insects here, there are so many. I'm going to kill the insects, they're insects, right? (The term 'insect' is used by some feminists to designate anti-feminists) ... I heard that

there were f*****g feminists here, I'm going
to murder them all (Chaigne, 2021).

While the attack, Haeil had to cover their bodies
and faces to protect their personal information
since New Men's Solidarity live-streamed the
whole situation. Hae-in Shim, a member of Haeil,
emphasized concealing their faces because the
cybercrime of collecting and posting personal
information, including photographs, is one of the
most common against feminists (Chaigne, 2021).

Furthermore, in an article named "Multi-layered
gender perception among young men," the author
indicates that young female feminists are aware of
daily misogyny and sexism. In contrast, a number
of young males disagree with the context of
structural sexism and strengthen anti-feminism
(Choo, 2021). With the subjects of 3,435 males aged
19-34 in South Korea, Choo (2021) categorized
participants into four types: request of
conservatism, transformation, sameness, and
acceptance of gender equality as a norm.

Figure 1: Ratios of four types
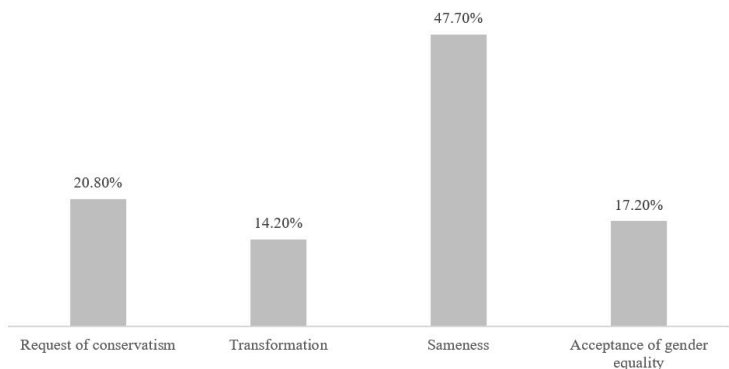
The "sameness" type (47.70%) opposes expanding legislation that would enhance the opportunity for women's involvement since they regard that gender relations are currently equal. The author claims that while this type may seem to reflect anti-feminism, they advocate expanding the laws against workplace harassment and violence against women. The "request of conservatism" type (20.80%) is opposed to both passive and proactive sexist policies. According to the study, this type tends to refuse traditional gender roles and norms but views women as immature, whom males should protect rather than compete with. Additionally, this type opposes change and argues that the current system of oppression of women is reasonable. The "acceptance of gender equality as a norm" type (17.20%) supports the expansion of all gender equality laws, including proactive measures to combat sexism. Nonetheless, this type is highly resistant to paternity leave and males' participation in housework, care, and gender equality, having the greatest acceptance of traditional gender role standards. Choo (2021) concludes that this type, although rejecting changes in everyday life and views regarding gender role norms, typically

supports different gender equality initiatives. Finally, the "transformation" type (14.20%) is the last to reject conventional gender roles and think women are treated unfairly today. This type is the most welcoming to policies that combat sexism and protect and assist women, as well as governmental measures that can encourage males to improve and actively participate in reducing this inequality (Choo, 2021). This research demonstrates how feminism and women are now seen in South Korean society and suggest a broadened view of sexism and anti-feminism.

Reasons of Exclusion

A researcher also points out the irony that TERFs claiming biological sex are more legitimate than individuals' gender identity (H. Lee, 2019). The author analyzes that TERFs in South Korea define femininity as misogynistic and reflects gender stereotype; therefore, they "set the ultimate goal and reason of feminism as abolishing gender" (p. 179). Furthermore, they contend that transgender people perpetuate gender norms and stereotypes, in contrast to the 'escape the corset' campaign, which began in 2017 and became widespread in 2018 (H. Lee, 2019). According to a CNN article titled "Escape the corset: How South Koreans are pushing back against beauty standards" (Jeong, 2019), the name of the movement was inspired by feminists' 1968 Miss America beauty pageant protest, in which they threw away "bras, hairspray, makeup, girdles, corsets, false eyelashes, high heels." This movement seeks to reject South Korean society's ideals of beauty and the pressure to

uphold them (Kuhn, 2019). As a result, many women take part in the movement by cutting their hair short, forgoing cosmetics, and dressing comfortably rather than in short, tight ensembles. Eventually, those women were the targets of assault and abuse by anti-feminists who assessed them based on their appearance.

Specifically, An San, a female archer from South Korea, won three gold medals in the Summer Olympics in 2021. However, a New York Times journalist reported that An San and other female athletes with short hairstyles at the Olympics had to face many online comments accusing her of being a feminist because of her short haircut (Jin, 2021). For instance, "Are you sure An San isn't a feminist, … She meets all the requirements to be one" was one of the remarks on An's Instagram. The article suggests that a professor of social network analysis at the Korea Advanced Institute of Science and Technology, Wonjae Lee, stated, "High-profile figures are often targeted by anti-feminists in South Korea" (Jin, 2021). The anti-feminists have become more combative and severe with women, seeing feminism as a perilous idea. According to a researcher named Hyomin Lee (2019), the feminist community is exclusive, specifically for male feminists, sexual minorities, including transgender people, and other women who are out of radical feminist boundaries. H. Lee asserts that TERFs describe gender as socially and

culturally determined sex, and they think this emphasizes gender stereotypes concerning the exclusion of trans women. Consequentially, there is tension between TERFs and transgender women because TERFs believe that many transgender women exhibit stereotypical femininity, such as wearing sexually suggestive clothing and having long hair. They view these are contrary to radical feminists' ultimate goal of gender dissolution and destroying the patriarchy, in accordance with the 'escape the corset' movement. In other words, TERFs in South Korea maintain that feminism and transgender rights are incompatible due to their conflicting stances (H. Lee, 2019).

Stances of Transgender People

Sarah McBride, a senator in Delaware of the United States and a spokesperson for the Human Rights Campaign, stated that TERFs view "deny the validity of transgender people and transgender identities" (McBride, 2020). Most feminists in the U.S., supporters of LGBTQ rights, and the mainstream medical community reject those views (Miller & Yasharoff, 2020). The American Medical Association (2018) emphasized that William E. Kobler, a board member, stated that sex and gender are much more complex than they are regarded and that restricted definitions of them may affect public health for transgender people and intersex people.

Personal Discrimination

Some people do not respect transgender people

and ridicule or attack them, and transgender people in South Korea face online discrimination. For example, a Twitter user once mocked trans people by posting, "I am a trans cat because I feel I am a cat. If you cannot understand me, you are a trans cat phobia" (H. Lee, 2019, p. 182). Likewise, H. Lee (2019) introduces several malicious comments and Twitter postings against transgender people in the article "Radical Reconstruction of Feminist Politics." According to the author, biology, notably sex chromosomes, is a major factor in TERFs' denial of transgender people's existence and their lives. The following YouTube comment prominently demonstrates the thoughts:

> The biggest dilemmas: 1) If you think you are a black person, can you be a black person? 2) If you think you are a cat, are you a cat? 3) If someone considers oneself an armless disabled person and tries to cut off their own arm, would it be an identity, not a mental illness? 4) Why ignore the basic scientific common sense that chromosomes determine sex? 5) What is a woman's mind/spirit? I will be a TERF until someone who can clearly answer these five questions appears. (H. Lee, 2019, p. 181).

In other words, TERFs believe that biology is the absolute fact and common sense, and they emphasize the unchangeability of gender from

birth, while they regard gender identity as an illusion. Since TERFs claim that "feeling" oneself as another gender regardless of the biological sex is illogical, they often mock transgender people; for example, belittling transgender people by setting the nickname "trans…" can be found online. Their central point is to stress that gender identity is an illusion and that the only and absolute fact is the biological sex from birth.

In February 2020, a trans woman student became qualified to enter a women's university in South Korea (Jang, 2020). Although she had already finished sex-change surgery in 2019 and successfully completed gender recognition, many enrolled students objected to her matriculation after learning of her admittance through news articles by calling the school's admissions office as a group and sending protest emails to the alumni association. Additionally, radical feminist clubs from more than 20 institutions created a social media account and published a statement against the student's enrollment and, saying, further opposing sex change that jeopardizes biological women's rights (Sookmyung Women's University T.F. Team X Against Transgender Male's Admission, 2020). One of the enrolled students mentioned the following in an interview:

> Women's college was created for women
> with fewer opportunities from birth than

men to education and social advancement.
… People who enter women's college are
those who were born as women and have
been socially discriminated against and
oppressed. I do not understand why a
person who has lived as a man until last
year wants to enter a women's college
(Jang, 2020).

On the other hand, several students did hold the
firm belief that in order for a society to be mature,
neither the institution nor the students should
exclude or denigrate any particular gender identity
(Jang, 2020). On Facebook, a statement supporting
the student claimed that South Korean women's
universities were founded on the ideology of
granting social minorities, particularly women, the
same rights to education (The Student and Minority
Human Rights Commission of Sookmyung
Women's University, 2020). In other words, the
commission determined that the fundamental
purpose of women's institutions is to address
prejudice and pursue equality. Thus, they claimed
that rejecting a specific person's identity and
debating whether or not to allow her admission
was against the university's philosophy (The
Student and Minority Human Rights Commission
of Sookmyung Women's University, 2020). The
transgender student did, however, eventually
renounce attending the university on the very last
day for students to determine their enrollment

(Kwon & Kang, 2020). She confessed that she felt fearful and heartbroken all day long for being cursed after seeing every comment and response about her (Kwon & Kang, 2020). She also discussed another incident when she went to the office of education to register for the national college entrance exam while wearing a one-piece dress, typically considered a women's outfit. A staff member reprimanded her attire and advised her that it would be better to dress "normally" because, saying, other students might feel uneasy on the day of the exam. She eventually had to switch to wearing pants instead of skirts. Before giving up on getting into the university, she said in the interview that she would be dedicated to informing the public about how anybody can be a minority in specific circumstances to emphasize the significance of equality. She expressed her wish that society would embrace each individual more and said that if individuals did not want to face prejudice and contempt in any other situations when they are a minority, they should appreciate and respect minorities of different identities (Kang, 2020).

Legal and Systematical Discrimination

Research on transphobia and discrimination in South Korea was undertaken in 2020 by the National Human Rights Commission (NHRCK), which also revealed the struggles transgender individuals face daily (Hong et al., 2020). In an online poll, 591 South Koreans over 19 identifying

themselves as transgender were asked to discuss their experiences in nine different categories, including gender recognition. As a result, only 47 participants (8.0%) said that they had successfully completed the legal process of gender recognition, 28 people (4.7%) were making progress, and 508 individuals (86.0%) had never attempted it. The participants' failure to attempt gender recognition was attributed to three main reasons:

Figure 2: Causes of not trying gender recognition
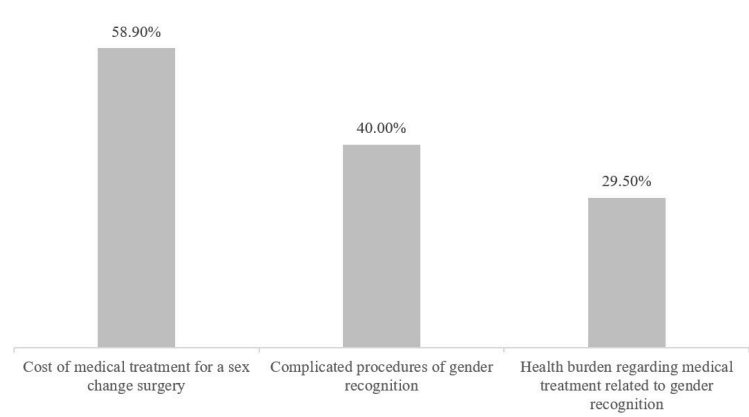
Furthermore, five critical factors were revealed by 82 subjects who have done or tried gender recognition:

Figure 3: Hardships during gender recognition

78.10%

61%

47.60%

31.70%

28.10%

| Meeting the requirements for medical treatment related to sex change surgery | Preparing the required documents | Finding accurate information | Difficulty in court hearing processes | Family members' opposition |
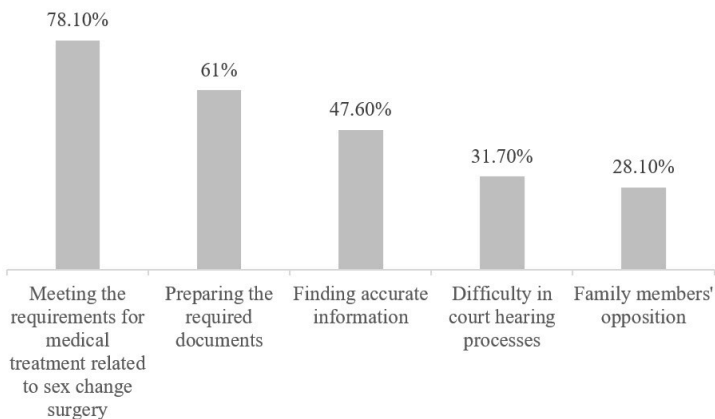
Finally, 78 participants answered that they encountered unfairness as a result of the gender recognition procedure (Hong et al., 2020):

Figure 4: Injustice during the gender recognition process

20.50%

10.30%

7.70%

3.90%

| Received sexually harssing/insulting questions from the judge during interrogation | Were required for unncessary documents | Court hearings were delayed for more than a year without any contact | Were requested to withdraw the application |
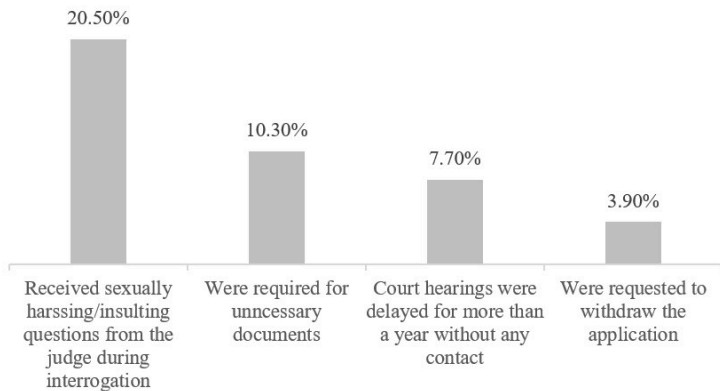
Although South Korea does not currently have a law addressing the gender recognition of transgender people, there

are guidelines that fall under Article 104 of the Act On Registration Of Family Relations (Rectification of Inadmissible Records Entered in Family Relations Register) (Korean Legislation Research Institute, 2019). According to the Supreme Court of Korea's guidelines, the following documents are required or optional:

- A certificate of identification documents
- A psychiatrist's medical certificate or written appraisal that identifies the applicant as a transgender patient
- A letter of opinion from a sex-change surgery doctor that confirms that the applicant currently has a contrary appearance (i.e., look like) to biological sex
- A medical certificate or written appraisal verifies that the applicant does not currently have a reproductive ability and is unlikely to occur or recover in the future
- The applicant's growth environment statement and acquaintance's warranty (Supreme Court of Korea, 2020)

A tragedy involving legal and systematical discrimination occurred in March 2021 when Huisu Byeon, a trans woman, took her own life at the age of 23 (W. Kim, 2021). She became aware of her gender identity during Korean mandatory military service and was advised to get surgery by the Armed Forces Capital Hospital after being identified as having significant gender dysphoria (W. Kim, 2021). Ultimately, she gained approval for a furlough overseas and underwent sex change surgery (Yu, 2021). She wanted to stay in the military

because her dream was to become a soldier. Still, the Discharge Review Committee forced her to discharge from the army in January 2020 due to a mental and physical disorder, suggesting the loss of her phallus and testicles as evidence of her "disorder" (Yu, 2021). The army adhered to the decision even though the NHRCK urged the military to postpone the review for three months. Byeon subsequently petitioned the Daejeon District Court to object to the discharge on August 11, 2020. Six months after Byeon's lawsuit was filed, the court set the first hearing date for April 15, 2021, after the U.N. sent a document to the Korean military stating that forcing her discharge violated international human rights law. The Military Human Rights Center's secretary-general, Hyungnam Kim, expressed his sorrow at Byeon's suicide by saying the following:

> I often resented the judiciary. Byeon could not even stand in court once because the court dawdled. ... When the military (the executive branch) disregarded Byeon, both the court (the judicial branch) and legislative branch, who were not interested in enacting anti-discrimination laws, joined the silence (W. Kim, 2021).

On March 16, 2021, at the National Assembly Defense Committee, the Minister of National Defense articulated the need for studies on the military service of transgender persons after Huisu Byeon's story gained widespread public awareness (W. Kim, 2021).

Female-Only Spaces

Definition & Necessity

Sheila Jeffreys, a well-known radical feminist in the United Kingdom, studied separatism and stressed the value of places exclusively for women (Jeffreys, 2018a). The formation of female-only areas is the most fundamental type of separatism, according to Jeffreys, who claimed that the Women's Liberation Movement (WLM) in the U.K.'s early 1970s applied the concept of separatism. She even asserted that "any attempt to create women-only space is challenged and sometimes threatened by men's rights activists and men who transgender" (Jeffreys, 2014, as cited in Jeffreys, 2018a, p. 55). The Free Space leaflet from the very beginning of the WLM organization in the U.S. declares, "We do not allow men in our movement because in a male supremacist society, men can and do act as the agents of our oppression" (Allen, 1970, as cited in Jeffreys, 2018a, p. 57). She also underlined the need for women-only places since Jeffreys (2018b) expressed fear that women would be unable to express their challenging ideas in the presence of males, the oppressors who can interrupt, become agitated or threaten them. In this context, Jeffreys emphasized the necessity for a safer location, contending that women are more likely to experience male violence in settings with mixed genders (Romito, 2008, as cited in Jeffreys, 2018a). She asserted that the premise for advocating for female-only spaces is to define "women" as a

political category created by patriarchal oppression resulting from living as a woman in a society where men are in charge (Jeffreys, 2018b).

Korean Female-Only Spaces

Various women-only facilities are already sprouting in South Korea, including parking areas, lounges, taxicabs, libraries, and fitness centers. However, there are differing views on whether or not certain areas are for women's safety and whether they constitute reverse discrimination against males. The supporters claim that the rates of sex crime against women are considerably greater than those against males. According to the report of the Korean National Police Agency (2021), 20,041 out of 21,717 sex crime instances involved female victims, which was 92.29% of the total. A news article stated that women-only facilities are appropriate for women's protection and claimed to have insights about their objectives by suggesting the statistics that around 40.27% of sex offenses occurred in environments that were known to everyday life (Democratic Press Citizens' Union, 2019). According to an article named "Women-only Space" (Ko, 2016), the construction of these places is due to visualized women's social achievements in Korean society in the late 1990s. Females' social successes—for example, passing national examinations, enrolling in colleges, and finding employment rather than staying home to care for the family—were seen as men's struggles or crises.

Since people blamed feminist movements and even all women in these critical situations, Ko (2016) explains that women-only spaces were one tactic to maintain women's voices facing harsh surroundings. In particular, the female student lounges in several coeducational universities in the capital region started to take shape in the late 1990s and early 2000s in response to demands of female students, which were prompted by common issues such as exclusion from cultures where men predominated and the need for networks as empowered spaces (Ko, 2016).

On the other hand, one particular case displays the idea that males are subjected to reverse discrimination due to female-only areas or services nowadays. In Jecheon-si, South Korea, there was a dispute in 2021 about the women-only library (S. Lee, 2021). With all the money she earned from sewing, an older woman named Hak-im Kim purchased and donated the land for the Jecheon Women's Library, which opened in 1994. Her goal was to resolve the discrimination against women seeking higher education. However, a man in his twenties claimed to the NHRCK in 2011 that it discriminates against men, and the commission urged the city and the library twice the following year. The city argued that the women-only library adheres to the donor's wishes and is irrelevant to sexism. Jecheon-si also elaborated on Kim's satisfaction and pride in the library during her

lifetime, as well as her family's hopes to continue operating in accordance with its original purpose. The NHRCK, nonetheless, determined that putting the donor's personal will ahead of a public facility's intended use is challenging since the national property legislation specifies that the donor's objective has no bearing on the law. The city argued in response that the library did not entirely exclude males because men are allowed to borrow books with the assistance of the staff, boys under the age of 10 can use the facility with a female caregiver, and the library has plans to have a family reading program. Yet, the NHRCK rejected such assertions and concluded that the library excessively restricted men's usage since there are no men's restrooms and men are not permitted in the material rooms. Although there is a city library close to the women's library, the commission claimed significant disparities in proximity while utilizing public transit. In the end, the library partially complied with the commission's exhortation and has let men borrow books since July 2021, but a library official the library is not considering additional actions, such as constructing men's restrooms (S. Lee, 2021). This specific incident indicates the ongoing argument about women-only facilities.

Fear of Invasion of Female-Only Spaces by Men

Regarding the aforementioned incident of the

trans woman student and women's university, a student who was against the enrollment stated in one interview that she felt uncomfortable after a series of events in males intruding on campus (Jang, 2020). She described two instances: one in which a male drug offender hid in the university's student union restroom and the other in which a man trespassed on a school lavatory while dressed like a woman. Even if there are no exact statistics, B. Lee and H. Kim (2020) describe a few particular incidents demonstrating how women's universities in Korea have been at risk from strangers' invasions. Specifically, a male student from another university broke into Sookmyung Women's University in April 2017 and was later detained on suspicion of sexual harassment. Also, after a male stranger engaged in indecent behavior while unclothed in a lecture room in October 2018, Dongduk Women's University barred unauthorized visitors. Moreover, Ehwa Woman's University increased the installation of security cameras and card readers at the entrance of the buildings following the accusation that a male office worker broke into the campus and touched a sleeping female student's body. Thus, several women's institutions decided to restrict visitors after encountering hidden camera crimes or males trespassing into the facilities (B. Lee & H. Kim, 2020).

In this context, Jeong (2018) claims that not just

online sexism but also daily sexual abuse has led to the desire for a safe space exclusively for women. Furthermore, radical feminists in South Korea have experienced high levels of sexual stress due to pervasive sex crimes, according to Jeong's (2018) analysis. This high sexual stress is caused by men invading women's spaces due to misogyny and sex crimes, including hidden camera crimes and the murder case at the Gangnam station. This claim corresponds to the abovementioned analysis that the "biological women" category discourse was constructed as a defense rather than to exclude trans women. Thus, the author examines that women in South Korea demand women-only spaces for safety and the power to voice and resist unreasonable misogyny today (Jeong, 2018).

Conclusion

Regardless of conflicting stances, it is true that both transgender people and feminists are those who should be respected. Due to concern for their safety and other factors, TERFs in South Korea began to reject transgender people. They refuse to embrace trans women as females whom they should protect by feminist goals for women's rights. However, although the exclusion has started to protect themselves, it is true that transgender people, in this study notably trans women, have experienced personal and institutional exclusion and discrimination. Both males and females otherize transgender persons and alienate them

from society. By setting stricter standards for other social groups, which likewise divides individuals, the discord between two groups may ultimately lead to increased and expanded exclusion. Feminism is a vital social movement for women's rights, but it is crucial to resolve the conflicts rather than excluding others based on rigid criteria for the category of women. Thus, reconsidering the definition and standards of females may help accept individuals to make a mature and welcoming society for every person.

References

American Medical Association. (2018, November 13). AMA adopts new policies at 2018 INTERIM Meeting. American Medical Association. https://www.ama-assn.org/press-center/press-releases/ama-adopts-new-policies-2018-interim-meeting

Bowcott, O. (2019, December 18). Judge rules against researcher who lost job over transgender tweets. The Guardian. https://www.theguardian.com/society/2019/dec/18/judge-rules-against-charity-worker-who-lost-job-over-transgender-tweets

Chaigne, T. (2021, September 14). The South Korean men waging a vulgar and violent war against feminists. The Observers – France 24. https://observers.france24.com/en/asia-pacific/20210914-the-south-korean-men-waging-a-vulgar-and-violent-war-against-feminists

Choo, J. (2021). Cheong-nyeon Nam-seong-deul-ui Jen-deo In-sig Da-cheung-seong [Multi-layered gender perception among young men]. Han-Gug-Yeo-Seong-Hag [Journal of Korean

Women's Studies], 37(4), 155–193. https://doi.org/10.30719/jkws.2021.12.37.4.155

Democratic Press Citizens' Union [민주언론시민연합]. (2019, September 16). Yeo-seong-jeon-yong-si-seol-i saeng-gyeo-nan maeg-lag-eul i-hae-hae-ya [We Need to Understand the Context of Women-Only Facilities]. Media Today. http://www.mediatoday.co.kr/news/articleView.html?idxno=202364

Gug-ga-in-gwon-wi-won-hoe [National Human Rights Commission of Korea], Hong, S., Kang, M., Kim, S., Park, H., Lee, S., Lee, H., Lee, H., Jeon, S., Kim, R., Mun, Y., Eom, Y., & Ju, S., Teu-laen-seu-jen-deo Hyeom-o-cha-byeol Sil-tae-jo-sa [Transgender Hate Discrimination Survey] (2020).

Jang, W. (2020, February 2). "Seong-jeon-hwan Nam-seong Ib-hag Ban-dae" Sug-myeong-yeo-dae-seo Hag-nae Ban-bal Um-jig-im [Sookmyung Women's University Protests Against Admission of Transgender Woman]. Yonhapnews. https://www.yna.co.kr/view/AKR20200201046200004?input=1195m

Jeffreys, S. (2018). Lae-di-keol Pe-mi-ni-jeum [Radical Feminism]. (Y. Kim, H. Nam, H. Park, Y. Lee, & J. Lee, Trans.). Yeolda Bukseu.

Jeffreys, S. (2018). The Lesbian Revolution: Lesbian Feminism in the Uk 1970-1990. Taylor & Francis Group.

Jeong, S. (2018). Geub-jin pe-mi-ni-jeum-eul kwi-eo-hyeom-o-lo-bu-teo gu-hae-nae-gi: Yeo-seong-un-dong-gwa seong-so-su-ja un-dong-ui yeon-dae-leul wi-han si-lon [Rescuing Radical Feminism from Queer Hate: A Theory for the Solidarity between the Women's Movement and the LGBT Movement]. Mun-Hwa-Gwa-Hag [Cultural Science], (95), 50–73.

Jeong, S. (2019, January 12). Escape the corset: How South Koreans are pushing back against Beauty standards. CNN.

https://edition.cnn.com/style/article/south-korea-escape-the-corset-intl/index.html

Jin, Y. Y. (2021, August 30). She just won her third gold medal in Tokyo. Detractors in South Korea are criticizing her haircut. The New York Times. https://www.nytimes.com/2021/07/30/sports/olympics/an-san-hair.html

Kang, J. (2020, February 2). Sug-myeong-yeo-dae Cheo-cho Teu-laen-seu-jen-deo Hab-gyeog-saeng "Ma-eum Neo-deol-neo-deol-hae-jyeoss-da" [Sookmyung Women's University's First Passed Transgender Student "My Heart Is Tattered"]. The Hankyoreh.   https://www.hani.co.kr/arti/society/society_general/926586.html

Kim, R.-N. (2017). Me-gal-li-an-deul-ui 'yeo-seong' beom-ju gi-hoeg-gwa yeon-dae: "Jung-yo-han geon 'nu-ga' a-nin u-li-ui 'gye-hoeg'i-da." [The Megalians' Project of the Category of Women and the Solidarities: "The Important Thing is not 'Who' but Our 'Plan'."]. Journal of Korean Women's Studies, 33(3), 109–140. https://doi.org/10.30719/jkws.2017.09.33.3.109

Kim, W. (2021, March 21). "Sa-beob · Ib-beob · Haeng-jeong-bu Mo-du Byeon-hui-su-leul Oe-myeon-haess-da" [The Judicial, Legistlative, and Administrative Governments All Turned a Blind Eye to Byeon Huisu]. The Kyunghyang Shinmun. https://www.khan.co.kr/national/national-general/article/202103210807011

Ko, Y.-K. (2016). Yeo-seong-jeon-yong-gong-gan [Women-only Space]. Yeo/Seong-i-Lon [Journal of Feminist Theories and Practices], (35), 167–180.

Korea Legislation Research Institute. (2019). Act On Registration Of Family Relations. Dae-han-min-gug Yeong-mun-beob-lyeong [Republic of Korea Law in English]. https://elaw.klri.re.kr/kor_service/lawView.do?hseq=54145&lang=ENG

Korean National Police Agency, 2020 Beom-joe-tong-gye [Korean Police Crime Statistics in 2020] (2021).

Kuhn, A. (2019, May 6). South Korean women 'escape the corset' and reject their country's beauty ideals. NPR. https://www.npr.org/2019/05/06/703749983/south-korean-women-escape-the-corset-and-reject-their-countrys-beauty-ideals

Kwon, J., & Kang, J. (2020, February 7). Sug-dae Teu-laen-seu-jen-deo Hab-gyeog-saeng Gyeol-gug Ib-hag Po-gi "Sin-sang-yu-chul Deung Mu-seo-um Keoss-da" [Passed Student at Sookmyung Women's University Eventually Gave up Admission "I Was Scared of Personal Information Leakage and So Forth". The Hankyoreh. https://www.hani.co.kr/arti/society/society_general/927386.html

Lee, B., & Kim, H. (2020, February 21). Bul-an-i-lan I-leum-ui 'Hyeom-o' ... Teu-laen-seu-jen-deo Bae-je-han 'Teo-peu' Hae-bu-ha-da ["Hate" in the Name of Anxiety ... Dissecting "TERF" That Excludes Transgender People]. The Kyunghyang Shinmun. https://www.khan.co.kr/national/national-general/article/202002210600015

Lee, H. (2019). Pe-mi-ni-jeum jeong-chi-hag-ui geub-jin-jeog jae-gu-seong: Han-gug' TERF'-e dae-han bi-pan-jeog bun-seog-eul jung-sim-eu-lo [Radical Reconstruction of Feminist Politics: Focusing on the Critical Analysis of "TERF" in South Korea]. Media, Gender & Culture, 34(3), 159–223. https://doi.org/10.38196/mgc.2019.09.34.3.159

Lee, S. (2021, July 10). "Yeo-seong-jeon-yong-eun nam-seong cha-byeol"... Yeo-seong-deul bun-no-han in-gwon-wi gwon-go, eo-tteoh-ge bwa-ya hal-kka ["Women-Only Facilities Are Discrimination Against Men"... How Should We View National Human Rights Commission of Korea's Exhortation That Made Women Furious?]. The Women's News.

https://www.womennews.co.kr/news/articleView.html?idxno=213588

McBride, S. (2020, March 8). About Sarah. Sarah McBride. https://sarahmcbride.com/about-sarah/

Miller, R. W., & Yasharoff, H. (2020, June 9). What's a TERF and why is 'Harry Potter' author J.K. Rowling being called one? USA Today. https://www.usatoday.com/story/news/nation/2020/06/09/what-terf-definition-trans-activists-includes-j-k-rowling/5326071002/

National Center for Transgender Equality. (2016, July 9). Understanding transgender people: The basics. National Center for Transgender Equality. https://transequality.org/issues/resources/understanding-transgender-people-the-basics

News1. (2021, May 17). 'Gang-nam-yeog Sal-in-sa-geon' 5Ju-gi... "Jen-deo-gal-deung A-nin Seong-cha-byeol · Yeo-seong-hyeom-o" [5th Anniversary of "Gangnam Station Murder"..."It's Not Gender Conflict, It's Gender Discrimination and Misogyny"]. dongA.com. https://www.donga.com/news/Society/article/all/20210517/106987080/1

Online News Team. (2016, May 18). Gangnam-yeog han-bog-pan-seo "Yeo-ja-deul-i na-leul mu-si-haet-da" mut-ji-ma sal-in [A Random Murder Happened in Gangnam Station Saying, "Women have ignored me"]. The Kyunghyang Shinmun. http://sports.khan.co.kr/news/sk_index.html?www&art_id=201605181037003

Sookmyung Women's University T.F. Team X Against Transgender Male's Admission [숙명여자대학교 트랜스젠더 남성 입학반대 TF팀 X] [@smwuwomyn]. (2020, February 6). Yeo-dae yeon-hab seong-myeong-mun. [Yeo-seong-ui gwon-li-leul wi-hyeob-ha-neun seong-byeol-byeon-gyeong ban-dae-had-da]] [A statement from the association of Women's Universities. [We oppose gender recognition that threatens

women's rights]] [Image attached] [Tweet]. Twitter. https://twitter.com/smuwomyn/status/1225382782106386433

The Student and Minority Human Rights Commission of Sookmyung Women's University [숙명여자대학교 학생•소수자 인권위원회]. (2020, February 2). Si-dae-ui yo-cheong-e eung-dab-hal geos-in-ga, hyeom-o-ui pyeon-e seol geos-in [Will you respond to the demands of the times, or will you stand by the hate?] [Image attached]. Facebook. https://www.facebook.com/100175401452787/photos/a.122986409171686/140433374093656/

Sullivan, R., & Snowdon, K. (2019, December 20). JK Rowling under fire over transgender comments. CNN. https://edition.cnn.com/2019/12/20/uk/jk-rowling-transgender-tweets-scli-intl-gbr/index.html

Supreme Court of Korea. (2020). Seong-jeon-hwan-ja-ui Seong-byeol-jeong-jeong-heo-ga-sin-cheong-sa-geon Deung Sa-mu-cheo-li-ji-chim [Guidelines for Handling Affairs of Transgender People Applying for Permission for Gender Recognition] . Dae-han-min-gug Beob-won Jong-hab-beob-lyul-jeong-bo [Korean Courts Comprehensive Legal Information]. https://glaw.scourt.go.kr/wsjo/gchick/sjo330.do?contId=3226349#1642204985543

Yu, H. (2021, March 4). 'Seoung-jeon-hwan Gang-je-jeon-yeog' Byeon-hui-su Jeon Ha-sa Sug-je Nam-gi-go Tteo-na-da [Former Staff Sergeant Huisu Byeon "Forced Discharge Due to Sex Change" Passed Away Leaving Homework]. Yonhapnews. https://www.yna.co.kr/view/AKR20210303181600504?input=1195m

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49. Breaking the Circle: Puerto Rico After Hurricane Maria

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My research, Breaking the Circle, is an intersectional analysis of Puerto Rico following Hurricane Maria (Maria). I will examine colonial US rule legislation such as the Jones Act signed in 1917 gave Puerto Ricans US citizenship and draft eligibility, Act 22 a tax incentive law specifically for non-Puerto Rican individuals and businesses, and PROMESA an unelected board which has complete oversight over the repayment of Puerto Rico's sizable debt to the United States. Studying the Jones

Act alongside more modern legislation like Act 22 and PROMESA highlights the deep-rooted political relationship between the United States and Puerto Rico. The Jones Act, Act 22, and PROMESA all have a similar goal: to perpetuate US colonial influence over Puerto Rico. These legislations paired with environmental injustice created a political landscape that led to the displacement of Puerto Ricans on the island and a complete disruption of traditional patterns of migration known as el vaiven or circular migration (Duany 2002). While the title of this paper specifically indicates the disruption of the traditional circular migratory pattern between Puerto Rico and US, this paper seeks to highlight the historical issues which Puerto Ricans have faced from their colonial relationship with the United States and the shortcomings of the colonial systems and connect them to Hurricane Maria a catastrophic environmental disaster that only brought to the surface these institutional issues which resulted in the colonial necropolis seen today. I examine the issues that Puerto Ricans face due to their colonial relationship with the United States. These issues have been present since the establishment of the colony, with Hurricane Maria serving as a watershed event exacerbating them further resulting in environmental injustice, socioeconomic inequality, and mass displacement

of individuals. Puerto Ricans have essentially become a second-class citizenry.

I will briefly discuss these legislative acts because they assisted in the creation of the current political climate which prioritizes US control and the perpetuation of colonial rule resulting in second class citizenry. The Jones Act bestowed Puerto Ricans US citizenship and draft eligibility. This act gave Puerto Ricans some autonomy in terms of governance and legislation, but they remain completely under US authority and rule. This act serves as the foundation of the legal and economic dependency between the United States and Puerto Rico. Similarly, the Puerto Rico Oversight, Management, and Economic Stability Act (PROMESA) was signed and mandated into law by President Barack Obama to oversee the payment and management of Puerto Rico's sizable debt of around 72 billion in 2015 (Mora 2019). PROMESA, locally known as La Junta, has cut spending in all public sectors with the goal of paying back this debt. PROMESA jeopardizes Puerto Rico's sovereignty as a Free-Associated State because La Junta has been given full power over the management of Puerto Rico's funding and in the 2023 court ruling of Financial Oversight and Management Board for Puerto Rico v. Centro de Periodismo Investigativo, La Junta was granted sovereign immunity. This means that PROMESA has unrestricted power over the island territory's

budget. Finally, Act 20, also known as the Export Services Act, incentivizes foreign businesses (more frequently mainland United States businesses) or individuals who have a high income to bring their assets to the island in hopes of stimulating the local economy. These incentives, however, are specifically for non-Puerto Ricans, and following the aftermath of Hurricane Maria, they were used by many developers and property owners who purchase substantial amounts of property on the island resulting in the displacement of many Puerto Ricans. The inclusion of these legal precedents serve to support my claim that because of local and federal mismanagement and outright political exploitation, the social, political, and economic inequalities seen on the island will only increase if the current trends are to continue.

Puerto Rico, following the ratification of the Puerto Rican constitution in 1952 was given the status of a Free-Associated State or Estado Libre Asociado which means that the island is given cultural and some economic sovereignty but remains under the direct governance of the United States. This makes Puerto Rico an anomaly not only in Latin America but among other nations. This relationship defines the political landscape on the island. The social, political, and economic relationship with the United States laid the foundation for the current situation on the island. Puerto Rico is situated geographically in the

Greater Antilles of the Caribbean, which makes it prone to natural disasters like hurricanes and tropical storms. Hurricane Irma, which preceded Maria, ravaged islands neighboring Puerto Rico. While the storm did not make landfall on the island, the winds and power-outages still impacted local infrastructure and was harbinger of the decimation which was to come. Puerto Rico's dependence socially, economically, and politically on the United States paired with its vulnerability to natural disasters left the island with an uncertain future.

The title of this paper, Breaking the Circle, is centered around circular migration, and how the disruption of this pattern has only exacerbated further the feelings of uncertainty that currently surround Puerto Rico's future. Jorge Duany coined the term, circular migration to describe the coming and going of Puerto Ricans from the US and the Island. This type of migration is a bit of an abnormality for other Latinx groups who have similar-sized communities in the United States. Puerto Ricans following the Jones Act granting US citizenship and the Great Migration of the 1950s have come and gone from Urban centers such as New York City in a circular or seasonal manner (Duany 2002). This type of migratory movement between the two territories resulted in what we typically understand to be a transnational nation. Puerto Rico serves as the cultural and familial home while places such as New York City served as a

temporary home where Puerto Ricans sought better-paying jobs in industrial sectors. This created an exchange of goods, ideas, and individuals between the Island and the Continental United States. Around the 2008 housing crisis, there was a similar economic crisis on the Island. La Crisis Boricua or Puerto Rico's severe economic crisis (Mora 2019) started around 2006 and ended around 2014 following several economic and political issues which led to a substantial drop in manufacturing jobs which resulted in Puerto Rico accruing a large national debt. This new economic instability laid the foundation for another large exodus, which could mirror or exceed the great migration of 1950. With the arrival of Hurricane Maria, there was mass economic and housing insecurity which resulted in an unprecedented exodus of Puerto Ricans to the US and a shift in the center of the Puerto Rican diaspora.

Figure 3. Estimated Migration Flows from Puerto to the U.S. 2005-2018
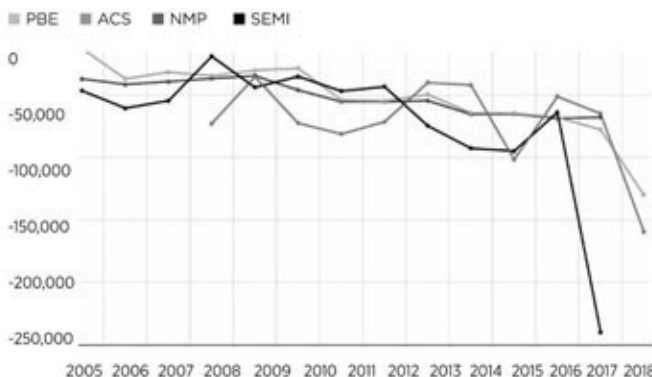
Source: 1941-2017 Department of Health and U.S. Census Bureau Population Estimates; 1991-2017 Bureau of Transportation Statistics; 2005-2018 American Community Survey; 2008-2018 Puerto Rico's Department of Education.
Note: NMP latest data available is September 2018 and PBE's vital statistics is unavailable for 2018.

(Hinjosa 2018)

This figure highlights the mass exodus of Puerto Ricans to the United States following Maria in 2017. By 2019 were an estimated 600,000 Puerto Rican migrated to the continental United States. With these new migrants, the center of the Puerto Rican diaspora shifted from the State of New York to the State of Florida. Not only did Maria change the landscape of the Puerto Rican population on the Island, but it also upturned the historical trends of the diasporic communities on the United States mainland.

Hurricane Maria in its wake left many homes, businesses, and communities destroyed. This created a chasm in which two figurative Puerto Ricos emerged. One is where communities and families are looking to rebuild, and another is a clean slate for businesses and individuals looking to make a profit. In the documentary, Landfall (2019),

this idea of two Puerto Ricos is explored in detail. One scene highlights a family in the mountains using their land to farm and rebuild while cryptocurrency billionaire Brock Pierce talks about the island being a place for economic opportunity and a haven of sorts for people like him. A journalistic piece done by a local news station, WAPA, titled Inversionismo del Desastre (2021) or disaster investment highlights the unequal purchasing of land on the island by developers who wish to capitalize on previously mentioned tax laws and economic and housing insecurity faced by Puerto Ricans. This emphasis on foreigners purchasing land in Puerto Rico is connected to the blank slate ideology that was used by the local Puerto Rican government immediately following Hurricane Maria to incentivize businesses and individuals who desired to come to the island and purchase land. (Whiteside 2019) The ideology of the phrase, "I live where you vacation" has been a marketing tactic paired with tax benefits that incentivized specifically mainland US citizens to move to Puerto Rico. This is unprecedented because historically Puerto Ricans have never been displaced on the island itself. Puerto Rico has been the cultural home for those on the island and in the diaspora. Because of the new laws and the disastrous effects of Maria, there has been a mass displacement of Puerto Ricans on the Island.

This reverse migration of sorts has been

supported and encouraged by local and federal governments. The migration changes following Maria have been categorized by unprecedented changes to traditional patterns in terms of centers of migration, migration to the island, displacement of local communities, and a mass exodus of Puerto Ricans. The feelings of uncertainty by those participating in this mass exodus are beautifully captured in the song, In My Old San Juan which tells the story of a man leaving his island behind to seek better opportunities without the certainty of returning and being able to return. He says, "Adios Mi Borinken Querida" meaning, "Goodbye my beloved Borinken (the name of the island in the Taíno language)." This sentiment, which is frequently expressed in the diaspora that speaks to the loss of "home" as a metaphor of displacement, is now commonplace on the island following Maria.

The word incertidumbre is directly translated as uncertainty but carries a more ominous meaning. The concept of incertidumbre is not something easily quantifiable but it is the daily reality for many Puerto Ricans on the Island. While abstract, incertidumbre highlights the harsh reality of facing and recovering from environmental, social, economic, and political crises. The issuance of Act 20 and PROMESA have further deepened this feeling because they further exacerbated economic and political instability. The feeling of incertidumbre is connected to the ideas of space,

place, and home. For those in the diaspora, incertidumbre comes from el vaiven the legal coming and going creates a transnational identity which for some, feelings of longing for their cultural home due to often forced separation because of economic insecurity. For Puerto Ricans on the island, the feeling of incertidumbre is connected to the uncertainty of the future of the Puerto they know and that which is yet to become. This idea of incertidumbre for those living on the island is explored clearly in the 2023 film titled La Pecera (The Fishbowl).

The protagonist Noelia serves as an incarnation of the generalized incertidumbre that prevails on the island. Noelia is diagnosed with late-stage colorectal cancer which is ravaging her body. We see Noelia, a Puerto Rican woman native to the Island of Vieques, suffering at her very core. Vieques served for many years as a US naval base for war games where live ammunitions and explosives were used. After years of protesting from the locals of Vieques, the US decided to abandon the naval base while leaving behind shrapnel, napalm, and polluted waters. The pollution left by the United States military has historically and presently been cleaned up by local groups. Noelia is one of these volunteers who has dedicated much of her life to cleaning up her polluted island. The cancer Noelia faces, and the cancer of colonial rule is a clear metaphor for the

current situation on the Island. The film highlights Noelia's journey in coming to terms with her sickness and it concludes before the arrival of both Hurricane Irma and Maria which are to arrive before Noelia's death. I conducted an interview with the director of the film, Glorimar Marrero Sanchez, and I asked her if the incertidumbre felt throughout the film was intentional because of the film's open ending, in which Noelia's death or the arrival of Hurricanes Irma and Maria are not explicitly represented. Marrero responded by saying the uncertainty of Puerto Rico's future and Noelia's future are similar. Noelia as a character is an allegory for the island's land and people. Her battle with cancer and the island's battles with systemic issues of neoliberal colonial rule and pollution are similar because death is almost certain but not yet fulfilled. This film validates and highlights the incertidumbre felt by Puerto Ricans because they are unsure and unable to control their future in terms political and legal sovereignty, environmental injustice, and economic stability. Incertidumbre has been at the core of the Puerto Rican life, in diaspora and on the island, it drives people away from their homes in search of a stable future. Hurricane Maria only further exacerbated these feelings and was a catalyst for the calamities that ravaged an already precarious situation.

Hurricane Maria made landfall on the southwest coast of Puerto Rico on September 20th, 2017.

Flooding, wind speeds of over 100 mph, and devastation to the already precarious electrical grid left many Puerto Ricans desolate. Some calculated the loss of life to be around two thousand people who died directly from the storm or because of the issues caused by the storm's catastrophic wake. Maria is a watershed event that took away the tropical island's beautiful exterior and laid bare the economic, environmental, social, infrastructural, and political issues that have historically plagued the island.

With the environmental destruction of the island, thousands of deaths, a decimated electrical grid, and a shortage of potable water, Puerto Ricans faced a harsh reality: to stay and rebuild or seek refuge in the continental United States. The island was in dire need of aid from the federal government and the slow economic and humanitarian response solidified what many Puerto Ricans already understood: they are not a priority for the United States Government. The current situation in Puerto Rico is a clear example of the intersection of political corruption, environmental injustice, legislation that has left the island environmentally vulnerable and opened to predatory economic practices especially in real estate and access to necessary resources.

Water regulation and potability have been at all-time lows making Puerto Rico the place in the United States with the worst rate of drinking water

(Llórens 2019). Without access to necessary resources like usable water and electricity, people are left without options. The isolation from necessary resources was a common characteristic of post Maria life. The lack of shelter, water, electricity, and overwhelmed hospitals and public services only made worse the incertidumbre felt by those left in the wake of the storm. A physician who worked on the front lines of the relief effort described his feelings, and those of his cohorts as, "My residents were overwhelmed, not just from physical exhaustion but from our patients' stories and the difficult decisions we had to make. We are not trained in disaster management, so we had to draw on our own personal and emotional strengths in managing the situation, aiming to provide high-quality and efficient care while maintaining our professionalism, humanism, and empathy" (Zorilla 2017). Not only did the lack of relief result in a destroyed electrical grid and widespread housing and economic insecurity, but it also overwhelmed and emotionally exhausted its physicians and medical staff. Similarly in the documentary Landfall, a woman describes the essence of life after Maria as the daily incertidumbre that they faced not knowing when or where help would be coming from. Some communities did not get power fully restored until a full calendar year after the storm. The local and federal governments failed them.

Puerto Rico before the storm had a sizable debt of around $72 billion in 2015.

This debt despite an unprecedented natural catastrophe remained the focal point for US interests and had increased to over $100 billion following the aftermath of Hurricane Maria (Mora 2019). This focus on the debt as a characteristic of the exploitative nature of the colonial relationship between Puerto Rico and the United States. (Lloréns 2019). Llorens describes this relationship as "slow violence". She describes this idea further as a "steady accumulation of gradual, and often invisible, environmental harms endures by vulnerable individuals and communities during capitalist expansion and neoliberalism" (Lloréns 2019). The US has leveraged its colonial power over Puerto Rico and its vulnerable populations by privatization of public services like energy providers, indifference toward pollution as seen by the pollution left by the military bases in Vieques, climate change, and gouging budgets all in the name of the repayment of Puerto Rico's debt. This approach, however, is just a perpetuation of previous colonial models of extraction and isolation of colonial inhabitants. The post Maria environmental injustice, the house and economic insecurity crisis created a chasm on the island and figuratively. Out of this chasm, two Puerto Rico's emerged. One where Puerto Ricans struggle for survival in the wake of environmental, social,

economic, and political injustice; and another where those who are aligned with US interests see a chance for exploitation, privatization, and profit. While the coming and going of Puerto Ricans to the continental US is not a new phenomenon, but the circumstances of their arrival caused by forced displacement and permanent migration to the US in the post Maria era is groundbreaking. Lloréns describes Post Maria Puerto Rico as a failed colonial project and a necropolis. She argues,

> The current historic exodus from the island also indicates that the contemporary political and economic model of the US territory has collapsed under the weight of neoliberal dispossession, shifting from the so-called Free-Associated State to a colonial necropolis of second-class citizens who can freely move to 50 US states, as a decade-long economic migration overlaps with climate change refugees whose very survival was at stake in the months after Hurricane Maria (Lloréns 2019).

Hurricane Maria was a watershed event that unearthed the already existing issues on the island connected to environmental injustice, political corruption, migration patterns, economic insecurity, resulting in a humanitarian, economic, housing, and social crisis due to the long-established colonial policies and structures. The

shortcomings of local and federal governments to protect Puerto Ricans and support them following an environmental catastrophe served to only worsen the systemic issues which have resulted in the modern-day crisis seen on the island. This lack of support locally and federally only feeds into the reality of incertidumbre which is central to the lives of many Puerto Ricans. This paper contributes to Puerto Rican, Latinx, Caribbean, and Post-Colonial scholarship and illuminates the current situation in Puerto Rico.

Works Cited

Aldarondo, Cecilia, Hofmann Kanna, Ines, Alvarez-Mesa, Pablo, Long, Terra Jean, Negrón, Angélica, Blackscrackle Films, Presenter, Independent Television Service, Production Company, and Good Docs , Distributor. Landfall. 2020.

Duany. (2002). Puerto Rican Nation on the Move. The University of North Carolina Press.

Hinojosa, J. (2018). Two Sides of the Coin of Puerto Rican Migration: Depopulation in Puerto Rico and the Redefinition of the Diaspora. CENTRO: Journal of the Center for Puerto Rican Studies, 30(3), 230+. https://link.gale.com/apps/doc/A581024271/AONE?u=marriottlibrary&sid=bookmark-AONE&xid=76cdc6fe

Lloréns, & Stanchich, M. (2019). Water is life, but the colony is a necropolis: Environmental terrains

of struggle in Puerto Rico. Cultural Dynamics, 31(1-2), 81–101. https://doi.org/10.1177/0921374019826200

Mora, Rodríguez, Havidán, & Dávila, Alberto E. (2021). Hurricane Maria in Puerto Rico : disaster, vulnerability, and resiliency.

Santiago, A. (2021, October 13). Inversionismo del Desastre. WAPA.TV. https://wapa.tv/programas/cuartopoder/inversionismo-del-desastre/article_af00caa6-beb0-5d3b-b161-f0bcd9e2dfa9.html

Whiteside. (2019). Foreign in a domestic sense. Urban Studies (Edinburgh, Scotland), 56(1), 147–166. https://doi.org/10.1177/0042098018768483

Zorrilla. (2017). The View from Puerto Rico — Hurricane Maria and Its Aftermath. The New England Journal of Medicine, 377(19), 1801–1803. https://doi.org/10.1056/NEJMp1713196

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50. Tennis Players and Bowlers: The Historical Sociology of the CIA

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Early CIA case officers recruited during the 1950s were characterized by similar upper-class backgrounds: wealthy families from the Northeast, preparatory school education, a degree from an Ivy League, and previous employment in white collar occupations. Anecdotal evidence reported that the next generation of case officers, recruited in the 1960s-70s, disrupted this stereotype: they came from working-class families in the Midwest and worked blue collars jobs. In interviews, some of these men have reported a cultural divide that separated them from their supervisors and predecessors. Based on data drawn

from memoirs, biographies, interviews, and congressional hearing records, there is a demographic shift between the generations, but it is less dramatic than reported. The second generation sees a greater influx of the middle class as opposed to the upper class, a transition rooted in external sociopolitical and ideological factors from the Cold War. Egalitarianism expresses itself as greater opportunities for individuals with working class backgrounds and an expansion of the middle class, but not as a breakthrough of the working class into the sphere of the elite.

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51. **"Epistolary Quackery:" 19th Century Cholera Discourses and Medical Professionalization**

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Abstract

In 1832, cholera appeared in London for the first time. This caused social upheaval and various actors with differing and often conflicting agendas competed for authority over the distribution of information to the public. Editorials and correspondence published in the Lancet and the

Times, a medical journal and a major newspaper, reveal these conflicts. In the absence of medical licensing, the editors of the Lancet sought to resolve issues of the legitimacy of medical knowledge within the journal's pages so the emerging profession could present a united front to the public. These efforts were undermined by practitioners who published their opinions on contagion or the results of therapeutic trials directly in the pages of newspapers such as the Times. The Times portrayed itself as a mediator of medical knowledge, but also used the discord that existed within the profession to advance its own political agenda. By the final major cholera outbreak in London in 1866, however, greater consistency existed between the medical information presented in both the Lancet and the Times. This is largely due to the integration of medicine into the government and the medicalization of public health.

Introduction

Cholera first arrived in London in 1832, in the midst of momentous social and political upheavals for Britain. The 1832 Reform Act, which received royal assent in June of that year, marked the culmination of campaigns for parliamentary reform throughout the 1820s and resulted in the extension of franchise to men who owned or rented land with a £10 yearly value or more. This expanded the vote

from about twelve percent to twenty percent of the adult male population, including many members of the urban, middle class businessmen who had previously not met property qualifications. With the passage of the act, many seats from rotten or pocket boroughs were transferred to manufacturing cities, leading to an influx of Members of Parliament who were invested in urban manufacturing and a laissez-faire labor market. While wealthier men benefited under the 1832 Reform Act, lower middle class and working class individuals continued to advocate for further expansion of suffrage and reform to electoral systems, which would eventually coalesce into the Chartist movement in the later 1830s.1

The struggle for democratization and deconstruction of old aristocracies apparent in the general political realm was also reflected within the medical profession. The regular medical profession consisted of physicians, surgeons, and apothecaries – these groups will be collectively referred to as "medical practitioners" in this paper. Prior to 1858, there was no centralized licensing system for medical practitioners in Britain. Instead, practitioners were licensed by corporate bodies for their respective professions; in London, these were the Royal College of Physicians, the Royal College of Surgeons, and the Worshipful Company of Apothecaries.2 Physicians practiced primarily among the rich and portrayed themselves as

gentlemen, while surgeons and apothecaries were tradesmen whose skill lay primarily with their hands, not their minds.3 In the early nineteenth century, divisions within the hierarchical tripartite system were beginning to break down with the rise of 'general practitioners' who practiced in all three areas.4 The medical colleges had reputations for being highly exclusive, with the College of Physicians and the College of Surgeons both being reluctant to recognize qualifications from provincial medical schools.5 In response to this, a medical reform movement began to emerge that sought to challenge the dominion of elites in the Royal Colleges within the profession and raise the status of medicine in the eyes of the public through regulation of unlicensed practice. Thomas Wakley, the editor of the Lancet, played a prominent role in this movement and was himself a political radical and Chartist supporter, though medical and political reform did not always go hand-in-hand for many medical practitioners.6

While changes were afoot within the medical profession, the same was happening in regard to medical knowledge. The rise of hospitals in the eighteenth and early nineteenth century helped develop the idea of localized disease pathology. Working in hospitals, practitioners were able to observe symptoms of disease in large numbers of patients, compare these cases, and observe the natural history of a disease with less obligation

to demonstrate the effects of their treatments than when working for private patrons. In this approach, the patient as a whole and as a person became increasingly obscured and diseases were viewed as afflicting particular organs or tissues.7

By the 1840s, this view of pathology would also influence disease etiology with zymotic theory. Detailed by Justus von Leibig in 1839-40 and adopted by William Farr of the General Register Office as an official disease classification in 1842, zymotic diseases were believed to be caused by specific poisons that caused ferment in the body which manifested as specific diseases. For example, the disease state of smallpox was said to be caused by transformations caused by varioline matter.8 Poisonous matter, or "zymes," acted as catalysts for disease processes that could become active under certain environmental and bodily conditions. The zymotic theory of disease represented a chemical turn to older miasmatic theories. In miasmatic theories of disease, which date back to the Middle Ages, diseases were caused by foul air arising from putrefying or decomposing organic matter. Zymotic theory posited that zymes could arise from rotting organic matter and endure in the environment or be spread through human contact. According to Farr's 1854 classification of zymotic diseases, miasmatic diseases diffuse through air or water and arise from either human/animal matter or earth/plant matter while contagious diseases are

transmitted through contact between people.9 However, though miasmatic and contagious interpretations of diseases like cholera could be at odds, they were not necessarily contradictory. A disease could be both miasmatic and contagious, initially arising from the environment and, under certain conditions, being transmitted from person to person. This "contingent contagionist" view became the most common interpretation of cholera by the time the disease settled into England in the mid-century.10

It is within the context of political, professional, and scientific change that various agents with differing agendas would compete for authority over information during the oncoming cholera epidemics. In a period of medical professionalization and reform, the Lancet medical journal sought to increase the credibility of the profession in the eyes of the public by discouraging practitioners from engaging in medical discourse in the public sphere, thereby creating the appearance of accord and establishing an exclusive knowledge base. The Times initially acted as a foil to the Lancet, sometimes exploiting medical discord to further its own goals.

Methods

This paper will examine material published on the subject of cholera in the Times newspaper and the Lancet medical journal during three outbreaks of cholera in London: 1832, 1854, 1866. These were

not the only years in which cholera epidemics occurred, with 1848-49 also constituting a major period of cholera in Britain. However, 1832 was the first year that cholera appeared in London and 1866 was the last major outbreak in the metropolis. 1854 is the best known cholera outbreak, perhaps of all time, as it was during this period that John Snow traced a local outbreak to the Broad-street pump. Along with this, it represents a mid-point between the 1848 Public Health Act and the 1858 Medical Act, two important pieces of legislation that impacted public health and medical professionalization. The specific timeframes examined were February through August of 1832, August through September 1854 (April through October 1854 for the Times), and June through September 1866. Each of these periods begins before cholera was declared epidemic in London, often before cases were even being counted, and ends once the cholera outbreaks subsided, though lower case counts continued to trickle in after some of the periods surveyed. A shortcoming of this approach, therefore, is that it does not include publications reflecting on the outbreaks from months or years later. As the Lancet noted in 1866, "the time of the actual outbreak of cholera is often a season of panic, unfavourable to careful observation and dispassionate reasoning, and fertile with wild hypotheses and puerile suggestions."11 However, this can also be beneficial

as the heat of cholera outbreaks was a time when medical knowledge was most hotly contested and most urgently needed.

Newspapers and Medical Periodicals

The Times and the Lancet were among the most widely circulated publications of their respective types during the 19th century. However, neither the Times nor the Lancet can be viewed as "typical" public or medical periodicals (if such a thing exists). The Times was able to gain supremacy over news periodicals by the 1830s in part due to its exploitation of non-union labor and, by 1832, was given the epithet "the Thunderer" for its vocal support of the 1832 Reform Bill. The paper represented the rising power of the commercial middle class and held such sway that its role was vital in government turnovers in 1834 and 1855. By 1855, however, the Times' dominance over the public press was ended with the abolition of stamp duty, allowing other publications to undercut its price; it also no longer was the leader in news-breaking, with other papers outpacing it in the latter half of the 19th century. As Andrew Hobbs has noted, the extensive focus on the Times in many nineteenth century histories has often obscured the vital roles played by local and regional publications as well as non-daily publications.12 One cannot assume that beliefs presented in the Times reflect those of other newspapers in London, let alone the rest of Britain. The same can likely

be said about the correspondence contained in the Times, which came from a specific portion of the country's population which was largely middle class, professional, and male (though there are exceptions).

The Lancet was also an exceptional publication in the 19th century. In contrast with other medical journals, it was published weekly (not quarterly), was relatively cheap, and was more widely circulated than its competitors. From its first issue in 1823, the Lancet positioned itself as subverting the established medical hierarchy, stating that it would shunt the language of the Colleges in favor of "plain English." As Brittany Pladek has argued, this was less an invitation to lay readers than an emulation of radical populist tactics for challenging exclusionary governing practices – in Wakley's case, aristocratic medical colleges.13 Early in its run, the Lancet published lecture series by the surgeons Astley Cooper and John Abernethy (without the speakers' consent) with the respective aims of increasing accessibility of valuable information within the profession shedding a light on incompetence and nepotism. Abernethy decided to pursue legal action against the Lancet, something with which the publication was intimately familiar as it was involved in ten legal proceedings, mostly libel, during its first ten years of publication.14 The Lancet was notable among the emerging genre of medical periodicals for its

radical perspective, dramatic and often theatrical editorials, and its combative style.

In the late 18th century, medical journals in which practitioners could publish case histories, courses of treatment, and more began to emerge. As Roy Porter discusses, these publications arose in an age when questions related to medical knowledge and authority propounded. Practitioners questioned whether medical information should be freely communicated or kept behind a veil of privacy, with reformers like Thomas Beddoes arguing that medicine could only progress through fact-sharing. He advocated the systematic collection of medical facts and increased medical publication. Questions also arose relating to how accessible medical knowledge should be to the public: "Was the publishing doctor a public benefactor? Or was he a self-serving publicist? [...] how far should the public be au fait with medicine, with a view to self-help and informed decision making?".15 There were also issues relating to medical authority and authenticity in a world in which practitioners themselves were debating what constituted orthodox medicine and what was deemed quackery or empiricism. In early medical periodicals such as the Medical Transactions Published by the Royal College of Physicians in London, all authors were members of the titular professional organization; however, even then, the journal included a disclaimer stating that the

College did not vouch for the truth of any papers published therein. Porter contrasts the conditions of medical publishing in Georgian England with those of the Victorian era, which was characterized by "a heightened sense of professional esprit de corps and exclusiveness".16 However, as will be shown, the discourses surrounding 19th century cholera outbreaks demonstrate that many of the questions identified by Porter in relation to 18th century publishing continued well into the 19th century, with professional periodical editors decrying practitioners who published in newspapers as quacks and trying to harness contradictory practices into some semblance of medical orthodoxy.

Public periodicals, like the Times, were also undergoing a period of change throughout the 19th century. The average length of the Times quadrupled from about 4 pages in 1832 to 16 pages in 1866. The newspaper transitioned from reporting international news in a section titled "Private Correspondence," indicating that these correspondents were not backed by the Times to having a large network of "Our Own Correspondents" as well as "Special Correspondents" that the Times dispatched to the Crimean War of the Atlantic telegraph expedition. In 1866, the Times published a reflection on the reporting of the Napoleonic Wars sixty years before; it observed that "The sources of information

were often of a very doubtful description in those days, and the Government received no better or safer intelligence than came by private hands" – the Times had reported the British capture of Flushing two days before the news reached England by any other means .17 Similar statements could be made about military intelligence during the Crimean War when the Times editorial stated that, if the government relied on official dispatches rather than newspaper correspondents, "the worst informed men in Europe would have been the English Ministers."18 Stephanie Markovitz notes that the Times functioned as a public forum in reality, not only appearance, because it provided a space where private experiences were freely expressed and co-mingled with public voices.19 However, as indicated by the Times' statements about the "rapidity and accuracy" with which intelligence from the Austro-Prussian War was reaching the country in 1866, official dispatches were becoming more reliable. The mix of public and private correspondence persisted through this period, but the Times' editorial stance, at least in respect to medicine, was shifting to favor centralized and official governmental reports over private correspondence.

Cholera Discourse in 1832 and 1854

When cholera arrived in England in 1832, no consensus existed as to its etiology and most therapies adopted by medical practitioners were

experiential or drew from older practices. It was generally agreed that "Asiatic" cholera was distinguished by vomiting and "rice-water" diarrhea, diminished pulse, and cold, blue skin. However, controversy existed as to whether these symptoms set the new cholera apart from "English" cholera or whether fecal diarrhea ought to be considered part of the disease proper or a premonitory symptom. In the 21st century, it is accepted that cholera is an infectious disease caused by Vibrio cholerae that is generally transmitted through ingestion of water or food that contains the bacteria, often through contamination by feces. It is primarily treated through oral or intravenous rehydration therapy that aims at restoring lost fluids.20

The discourses that formed around 19th century cholera outbreaks reveal an aspect of medical professionalization sometimes passed over: the desire for consensus and uniformity of opinion presented to the public. This is repeatedly betrayed by Lancet editorials. In the 1832 outbreak, no agreement existed within the medical profession as to cholera's contagiousness or lack thereof – medical practitioners airing on both sides of the dispute were published on the pages of both the Times and the Lancet. However, the editors of the Lancet strongly believed that cholera was contagious, and branded practitioners who expressed anti-contagionist views in the public

press as attention seekers, chasing after ephemeral fame. It balked at men who leave their "proper spheres and exhibit their grotesque movements to the gaping public"21 – discussion of technical matters should occur within the medical sphere and should not be visible to the public. The Lancet's concern was partially caused by the harm that could be caused by public dismissiveness of the threat posed by cholera, but it was also motivated by the desire that the medical profession present a united front to the public. The inclusion of the public in medical discourse also perpetuated the notion that lay people were as qualified to pass judgement as practitioners, a notion harmful to the cause of professionalization which necessitated the creation of an exclusive knowledge base.

Throughout the period, the Lancet also advocated for the establishment of commissions or governmental inquiries to conduct large scale studies which, it hoped, would put an end to the "epistolary quackery" found in public papers and settle medical disputes.22 The editors disparaged medical "empiricism" characterized by individual practitioners developing therapies and theories of disease based on their personal experiences, rather than large scale trials. This perpetuated a network of medical knowledge that contained diverse and often conflicting ideas, decreasing the credulity and authority of any information issued by medical practitioners.

The Lancet's attitudes toward medical empiricism and pluralism were both borne out and informed by the Times to some extent. In 1832, the Times seized the lack of agreement among practitioners about contagion to push an anti-contagionist agenda favorable to its mercantile disposition. Similarly, the Times promoted the use of castor oil because of the lack of a clearly effective treatment for cholera and the myriad treatments advocated by medical practitioners. In both instances, the Times' approach provoked the Lancet to respond by calling for unity among medical professionals and separation of the spheres of medical and public discourse. The following case studies will provide further illustrations of these dynamics.

Case Studies

1. The Cholera "Humbug"

Throughout the 1820s, cholera traveled from the Indian subcontinent to Europe and Russia, with Britain watching every step of its progress. To prepare for the threat, the Office of the Privy Council gathered cholera intelligence from abroad and sought advice from the Royal College of Physicians. The President of the College deemed cholera contagious and recommended quarantine measures for cholera under the 1825 Quarantine Act. However, the College also gave a collective opinion that the disease was not contagious. To settle the issue, the Privy Council asked the President of the College to recommend members

to sit on a board of health that would advise the government. This temporary Central Board of Health was established in June of 1831 and was expressly aimed at addressing cholera. It recommended the formation of local boards of health to enact sanitary measures. Over the summer of 1831, the contagion debate raged. Contagionists won a victory when the government declared a fourteen day quarantine for vessels; however, anti-contagionists were fueled by testimony from medical practitioners who had seen cholera in India that it was not contagious. The debate about whether or not cholera was contagious was not just about medical theory, but also (and oftentimes primarily) about regulating commerce and labor through quarantine. Despite the initial enforcement of quarantine measures, cholera cases started to appear in northern England in October and November. From its initial appearance in Sunderland, the epidemic began to spread throughout Britain and would soon make its way to the capital.23

The Times reported London's first cholera cases on February 14, 1832.24 The same editorial in which the newspaper announced that cholera was present in London also directed the readers' attention to a letter by "Theta" published in that day's issue. This letter asserts that the cholera currently spreading in London and throughout England was not a new disease that originated in Asia, but was native to England and existed in the country in a milder form due to differences in climate. In the letter, Theta also accuses the Board of Health

and the medical profession of exaggerating the cholera cases and exciting public fears, stating that "The profit derived from cholera-phobia by the profession at large, who have obviously no interest in exposing the groundless nature of the alarm" explained medical practitioners' support for the "hoax."25 At this point, the Times still presented itself as neutral on the cholera question, stating that people should apply themselves to following healthful habits and leave "the men of science and political economists to dispute those matters which peculiarly appertain to their studies."26 The paper still placed some degree of credence and trust in official reports and the conduct of medical and scientific professionals. In fact, in an ironic twist, the Times published a letter describing how "mischievous" periodicals in the north had persuaded their communities that cholera was not contagious, with fatal consequences. The writer imagines what "indescribable horror" could occur if anti-contagionism took hold of London and says that it therefore "behoves you (the press) therefore to continue to put forth all your energies in enforcing the means of suppressing and averting this calamity."27

The Times would do exactly the opposite. The following day, the editorial puts forward the paper's official view that the cholera present in England was neither new nor Asiatic and cites correspondence from Dr. David Uwins and an unnamed Indian surgeon to support this claim.28 A day later, the Times printed a note in its "To Correspondents" section stating that "We cannot publish specifics for cholera sent by our numerous medical correspondents: no two of them agree."29 The newspaper utilized this lack of consensus and

the support of a few practitioners to advocate for the preservation of laissez faire trade and commerce in the face of cholera. The editors state that they support cleaning and sanitary efforts, but not any course of action that would interfere with trade. They justify this by arguing that curtailing trade would leave people unemployed and inactive, making them more susceptible to the disease, which was caused by "poverty, bad living, insufficient clothing, dirty streets and dwellings, united to occasional excess."30 As the Times was known for representing the interests of the commercial middle class, this opinion would have been popular among a substantial portion of its audience, many of whom were more directly involved in trade that would be affected by quarantine than in the lives of the poor who were the primary victims of cholera. However, the notion of a cholera "humbug" was also popular among the working class as it accorded with commonly held notions of governmental and professional corruption, with officials inventing cases to keep jobs paid by public taxes.31

The newspaper's editorial stance became more conspiratorial as the month advanced. Fears over cholera were not just exaggerated, they had been maliciously stirred up by interested parties. The Times' editorial also argued that cholera had been "got up" to distract from the reform bill before Parliament.32 It alleged that the medical profession

"has been brought into utter contempt by the system of charlatanism to which truth and common sense, and the comfort and welfare of society, have within the last three months been made victims."33 By the end of the month, the Times stated that "Official announcements have long ceased to be gospel with us."34

   On the pages of the Times from early 1832, one witnesses a landscape in which no official knowledge could go uncontested. The Times' editorial and correspondents pointed to the similarities between the Central Board's description of the new Asiatic cholera and descriptions of "cholera morbus" described in publications like Dr. Buchan's Domestic Medicine decades earlier. Both describe vomiting and purging, a low pulse, cramps, cold extremities, and urinary obstruction (though it should be noted that Buchan describes vomiting and purging of bile while the Central Board expressly states that evacuations are not bilious).35 Medical practitioners and laypeople alike frequently visited, or attempted to visit, hospitals or the homes of reported cholera cases to ascertain whether individual cases had been falsely reported.36 Medical practitioners frequently wrote in pointing out individual cases of under their treatment which they believed to have been misreported by overzealous colleagues.37 People who were suspicious of overreporting of cholera in certain

areas would also write to the paper to request that someone investigate and ascertain whether the reported cases actually had cholera.38 Another common theme was individuals with chronic conditions, such as "windy spasms," being wrongly reported as having cholera.39 This practice did not go uncriticized in the Times – Alex Tweedie wrote to the paper complaining that people "who, from their obscure station in the profession, would in any ordinary circumstance hardly be entitled to a medical opinion at all" making pronouncements after cursory visits to cholera wards.40

However, the Times framed its medical correspondents very differently, saying that the "ablest and most estimable physicians in London and in other parts of England have [...] declared the official manifestoes, assertions, injunctions, precautions, reports, and so forth, to be one mass of humbug."41 The Times, therefore, was the purveyor of estimable medical information in publishing reports of the cholera "humbug." In resolving that anti-cholerists and anti-contagionists are the most respectable medical practitioners, the newspaper decided that their medical views were accurate while those of other medical practitioners were not. It created a dynamic in which the Times was not choosing a side in a medical debate but was rather just conveying the opinions of the most established and reputable practitioners (a group which happened to exclude governmental officials). This

framing was necessary because, even though the editors very obviously had a stance on this medical issue, it was still a public paper with no pretense of possessing technical medical knowledge. Various correspondents lauded the Times for being "properly reluctant" to admit technical papers or expressed reluctance at writing to a public paper rather than a medical one.42 This demonstrates that medical practitioners who wrote to the paper still viewed it as occupying a separate sphere from a professional publication like the Lancet and that it was improper for its editors to become embroiled in professional disputes. Unlike the editors of the Lancet, the editors of the Times did not make an argument based on their own authority and knowledge; rather, they did so based on the authority of the medical correspondence they curated. This dynamic is perhaps most clearly articulated in the Times' statement that "We again commend to public notice the Report of the 'Central Board of Health' and at the same time other documents by which that report is contradicted. […] We are not medical people, and our concerns are in no way whatever affected by the existence or non-existence of the cholera."43 The Times portrayed itself as a neutral arbiter of medical information, only relaying the views expressed by the respectable majority of the profession. A person reading only the Times during early 1832 could easily have assumed that it was a

widely agreed-upon fact that cholera was not a new or contagious disease in England.

The Times' easy course into the realm of conspiracy is indicative of the status of the medical profession at the beginning of the 19th century. As Roy Porter has argued, the 18th century was a time of great medical plurality, with "regular" practitioners practicing alongside a wide variety of "irregular" practitioners in a medical marketplace. There was little differentiation in status between regular and irregular practice and "quackery" abounded in both groups. Regular physicians' cures "were experienced as tediously protracted, expensive and disgusting, […] their demeanor often loathed as pompous, and patients commonly doubted their competence."44 This, combined with the facts that there was no governmental regulation of medicine and medical colleges did little to police the profession, meant that licensed medical practitioners were not viewed as holding status or authority that set them or their ideas apart from the myriad others found in newspapers. Their participation in a medical marketplace, characterized by self promotion and rife with quackery, would have primed the Times to identify self-interested motivations in practitioners' assertions that cholera was a serious threat. The Times did not view medical knowledge as authoritative or all of its practitioners as particularly noble parties, and, therefore, took it

upon itself to dispute and critique the actions and beliefs of medical practitioners when they clashed with the Times' interests.

While the Times sought to dispute medical knowledge, the editors of the Lancet attempted to consolidate it and argued that the rhetoric the Times engaged in was an insult to the medical profession. At the beginning of February, the Lancet published an editorial tracing the progress of cholera through England and Scotland. In it, they identify several cases clearly attributable to human communication and conclude that there is "irrefragable proof" that cholera can be spread via contagion.45 The Lancet editors, unlike the Times, were willing to "stake our professional and literary character on the assertion, that numerous cases of the malignant Asiatic cholera have occurred in London."46 This, they state, can be established through consistency in cause, symptoms, and pathology, with any two coexisting being sufficient to prove the presence of Asiatic cholera. With the cause of the disease still unknown, they go on to examine individual cases that have been reported as cholera, finding at least one case in London where both the symptoms and morbid anatomy coincided perfectly with Asiatic cholera (the body was also examined by a doctor who had served in India, lending credence to his judgement).47 This prime example of a cholera victim is described as having been afflicted with "a loose, irritable state

of the bowels," vomiting, cramps and spasms in the extremities, sunken and blueish features, oppressed respiration and pulse, and a cold tongue and skin. Upon dissection, he had a "whitish turbid fluid" in the intestines and his blood was dark and often congested, among other features.48 According to the positive burden of proof that the Lancet had established for itself, this was enough to demonstrate that malignant Asiatic cholera existed in London.

The Lancet's editorial position on the rhetoric surrounding cholera did not end with firmly asserting that Asiatic cholera was present in London and likely contagious. Rather, the editors set out an exhaustive critique of the positions taken by public papers, like the Times, and many of the medical men who decided to publish in their pages. The journal viewed this as its duty to both the public and the medical profession, stating that "never was a portion of the public more successfully, more dangerously, deluded, and never, in our sincere belief, was there a more scandalous bolt flung upon the dignity of British medicine."49 In examining the make-up of the "anti-cholera party," the Lancet identified "The Times and its tail, all that is notorious for stupidity and venality in the metropolitan press,"50 indicating that, while the Times was not the only purveyor of anti-cholera rhetoric, it was certainly an important one. The editors counter the Times' arguments with

historical data showing that the plague death counts mimicked those of cholera early in the epidemic and with a list of the emoluments ofmedical inspectors and members of the Central Board of Health, based on Parliamentary papers, to prove that the Times had grossly exaggerated the pay of these officials.51 The Lancet concludes that, "we trust we need say no more either of the erudition of the veracity of the journal in question, or of the right it has to direct the minds of rational and honest men."52 In this statement, the Lancet is commenting more on the Times' journalistic integrity than its ability to make statements claiming medical authority, largely because the Times was doing no such thing. However, the same could not be said for medical practitioners publishing in its pages.

The editorial goes on to address the "more disagreeable topic" of medical practitioners who denied the existence of cholera in London and aired these opinions in papers like The Times. The Lancet viewed itself as a defender of the medical profession, stating that, up until its founding, "the bulk of English medical practitioners were destitute of the protection afforded by an independent journal; the press, up to that day, had never been employed for them, but always against them."53 Protecting the medical profession often meant policing (or at least commenting on) which of its members functioned as emissaries to the public and

what the public perceived of the inner functioning of the profession. The paper was concerned with having "eminent" and "reputable" medical opinions conveyed to the public and, in its judgment, most anti-contagionists were neither.

The Lancet characterizes anti-contagionist medical practitioners as individuals who, in their false statements, had fallen from grace in the profession or only rose from obscurity through notoriety. The theme of medical practitioners unrightfully using the public press to gain fame and credence was one often repeated in its pages. While the "Times and its tail" characterized anti-cholerists they cited as "eminent men," the Lancet responded by describing them as "those persons whom the cholera has had the effect of calling into ephemeral existence which vain and weak, but busy men sometimes obtain by the publication of their names in the columns of a newspaper."54 These statements reveal a distrust of obscure medical professionals who decided to publish in the public sphere rooted in 18th century notions of quackery. These practitioners were not publishing in newspapers for the good of the public, greater accessibility of information, or faster dissemination of findings, but to garner unwarranted fame and attention. Despite the fact that anti-contagionists were not selling nostrums and some did not even provide their names in the Times, their actions were reminiscent of quacks who "proclaim their medical abilities in

public places."55 It also seems likely that certain practitioners receiving special editorial attention from the Times which would function to raise their public personas.

The Lancet editors did not believe that this type of behavior was appropriate and advocated for a separation of spheres of medical and public discourse. When the formation of an Association to Investigate the Cholera was announced, the Lancet commented that "if men will leave their proper spheres and exhibit their grotesque movements to the gaping public, who is to be blamed for the disgrace consequent upon the offence?,"56 indicating that debates among members of the medical profession should not occur in public forums. The "grotesque movements," once visible to the public, served only to discredit the profession. The editors added that their reaction would have been very different had the association included prominent chemists like William Brooke O'Shaughnessy and pathologists like Charles Bell. Correspondence from individuals whose "renown is of that quality which is created by an ephemeral puff, or a paragraph in a morning print" was more likely to be self-promoting quackery.57 Furthermore, they were more likely to shape their medical opinions to fit the beliefs of their clients to ensure further patronage, a common practice in Georgian medicine.58 In the case of the Times, practitioners might proclaim themselves as anti-

contagionists to fit the well-established editorial position of the newspaper, which was likely shared by its readership, some of whom might have been looking for a new physician. However, people like O'Shaughnessy and Bell already had established reputations and were therefore more impervious to this type of influence. It likely helped that O'Shaughnessy was a relatively frequent correspondent in the Lancet – endorsing him as a reputable practitioner was also the Lancet endorsing itself as the proper forum for intra-professional discussion.

However, the Lancet was not completely opposed to the dissemination of medical discourse in public papers. It supported the publication of medical society discussions in public papers as these had become the only means through which both sides of the contagion debate were available to the wider public.59 While some members of the medical profession viewed publication of these proceedings in widely disseminated papers as "puffing" and a form of extravagant promotion and advertisement, the Lancet argued that "it is the manner and the object of publicity, and not publicity itself, which constitute that art, at which some of the objectors themselves, by the way, are the most thorough adepts."60 The editors believed that publishing medical discourse in public papers, in and of itself, did not constitute "puffing." In fact, it was necessary for the public to have access to balanced medical

information. The true puffery was individuals of little repute seeking notoriety in the public papers.

It is notable that the Lancet, critical of elitist medical societies and nepotism, advocated for the publication of surgical and medical proceedings to encourage professional criticism and challenge the authority held by exclusive colleges early in its run.61 As a champion of medical reform, the Lancet advocated for increased transparency within the profession and allowing general practitioners to have access to the same information and opportunities as members of elite medical societies. In shedding light on these types of proceedings, the lay public would also be let in on the inner workings of the profession. The object of this publicity was not the aggrandizement of individual practitioners or advocacy of a particular nostrum or pathology; therefore, it did not constitute puffery. It also differed from the actions of groups like the Association to Investigate the Cholera in that the members of medical colleges and societies like the London and Westminster Medical Societies had established reputations and the publication of their proceedings was not a means of creating ephemeral fame. Despite the Lancet's opposition to the elitism of many medical societies, it relied on their reputation to legitimize medicine to the public while also opening them up to scrutiny from within the medical profession.

The Lancet seemed certain that once the upheaval

of the outbreak died down, practitioners seeking fame in the pages of newspapers would be revealed as attention-seeking falsifiers.62 However, at present, they were not necessarily considered persons of ill-repute in the wider medical sphere. In fact, Dr. James Johnson, who the Lancet identified as one of the leaders of the anti-contagionist party, was the editor of Medico-Chirurgical Review and a prominent member of the Westminster Medical Society whose correspondence the Lancet often published.63 One can infer that the Lancet was more concerned with the image of the medical profession in the public eye than restricting the discourse on its own pages. Even during the height of the cholera controversy in February and March, the Lancet published correspondence that argued that the disease was not new in England and that it was definitely not contagious.64 Outside of the Lancet's editorial section, these views were not even in the minority. This demonstrates that individuals who did not agree with the Lancet's editorial stance still sent correspondence to the paper and that the Lancet did not refuse to publish correspondence that did not accord with its views. It also shows that the Lancet was not concerned about contradictory statements within its pages, though the same could not be said for medical discussion in public papers.

While it seems likely that the Lancet exercised relatively little editorial discretion in publishing

correspondence related to contagion, it is unclear to what extent the Times engaged in such activities. Based on the Times' statement that "We continue to receive more letters on cholera than would fill our paper daily [...] We shall publish none, except such as contain some curious and well-established fact, or are written by some person of established reputation,"65 one can ascertain that the newspaper could exercise a large degree of choice in which correspondence to publish and which not to. The editors state that they will only publish correspondence with well-established facts or from reputable persons and, given that they already decided that anti-contagionists were the most reputable medical professionals, this creates the perfect groundwork for an echo-chamber in which the Times only included correspondence that repeated "facts" already established in the paper. However, the Times' published correspondence was not unanimously anti-contagionist and grew increasingly heterogeneous as the year progressed.66

The spring of 1832 was marked by a decline in both cholera cases and discussion of its contagiousness in the Lancet and the Times. When cases began to climb again in the summer, both publications' partisanship on the question of contagion softened. When commenting on the people being released from a prison affected by cholera, the Times editorial entertained the

possibility of the disease being contagious and voiced concern about it spreading through infection.67 The Lancet editorial, in turn, voiced the opinion that cholera was both epidemic and infectious and that "it is principally in places free from the epidemic that the disease can, under ordinary exposure, spread from person to person."68 While the shift in the Times' tone can be ascribed to its desire to not see prisoners released, the Lancet's strong contagionist stance was undermined by patterns of cholera infection. There were simply instances in which no person could be traced who could have transmitted cholera from one place to another, leading the Lancet to adopt a contingent-contagionist view. According to modern understanding of cholera, the reason for the Lancet's softening in tone can be attributed to the fact that Vibrio cholerae is generally vector-borne, rather than being transmitted directly from person to person, circumventing the need for direct contact.

2. The Saline Treatment

By the time cholera reached London in February 1832, various treatments had emerged among British medical practitioners, many of them informed by the experiences of colonial surgeons and physicians who had experience with the disease in India. Most agreed on various aspects of cholera pathology. One of the primary aspects of the disease is decreased circulation marked by

coldness of skin and a weak pulse in the appendages – some descriptions expand on this by detailing how blood is coalescing in the center of the body, diminished energy from the heart, or through thickening of the blood.69 Those who focused on changes in the circulation of the blood mainly prescribed bleeding or external applications of heat to draw the blood back towards the skin.70 Bleeding was by far the less common of these treatments, with most practitioners prescribing some form of external heat or poultice regardless of the other therapies they pursued (one recommended wrapping patients in the skin of freshly slaughtered sheep).71 Many medical practitioners also ascribed many of cholera's symptoms to disruption of the function of the liver. The cholera poison either increased the bile secretion of the liver, resulting in vomiting and purging which eventually led to collapse; or it suppressed bile secretion.72

The course of treatment that received the most attention in the Lancet was based in a pathological interpretation that viewed the evacuations as the cause of exhaustion associated with the final state of "collapse" in cholera. While many sought to treat this through the use of astringents, the "saline method" advocated for treating the effects of evacuations was based on analyses of the blood of cholera patients conducted by the Irish chemist and physician, William O'Shaughnessy. He found

that, in comparison with normal blood, the blood of those afflicted with cholera showed decreases in the amounts of water and soluble salts, which were instead found in patients' "rice water evacuations."73 Similar results were found by others who conducted analyses of cholera patients' blood. Based on this, various saline treatments emerged, which functioned as precursors to both modern oral rehydration therapy and intravenous therapy. Dr. William Stevens and Henry Wakefield, working in the Coldbath-Field Prison developed a treatment in which "the main reliance is upon the following powder, to be administered every hour in half a tumbler of cold water, viz: – Carb. Of soda half a dram; common salt 20 grains: oxymuriate of potash 7 grains."74 This is closely reminiscent of modern oral rehydration therapy (ORT) which involves oral administration of water combined with sugar and salts to treat dehydration due to diarrhea. In cholera, Vibrio cholerae disrupt the body's ability to reabsorb Na+ ions in the digestive tract. This changes the osmotic pressure of the intestinal tract, causing water to diffuse out of cells in the intestines and leads to diarrhea and dehydration. Modern oral rehydration therapy restores lost sodium and water to cells by using glucose to facilitate Na+ transport into cells.75 Given that Stevens' treatment did not include glucose, it would likely not have been as effective as modern ORT, but it likely would have helped

restore depleted sodium and water to some extent. Stevens, based on the blood pathology provided by O'Shaughnessy, reasoned that administering water and salts would restore the substances missing in the blood. Following a similar line of reasoning, Dr. Thomas Latta, joined by Robert Lewins and Thomas Craigie, advocated the use of copious injections of saline fluids into the veins. He observed that deficiency of water and saline matter in the blood caused "the thick, black, cold state of the vital fluid" which produced many of the effects of cholera and often directly resulted in death.76 This method of treatment has given Latta the epithet "the father of intravenous infusion therapy."77 In the present day, intravenous therapy is also considered an effective treatment for cholera, though less accessible than ORT.

The Lancet viewed these "saline treatments" as highly promising. To the editors, the saline treatments represented medicine based on an innovative combination of chemistry, pathology, and clinical trials and "teaches us how boldly we may proceed when certain and scientific data are before us."78 However, this optimism and excitement would not last. By June 23rd, the Lancet editorial threatened that, if Stevens et al. continued to conduct their experiments in the present manner, "the whisper of detraction will doubtless be heard in many corners [...] that Dr. Stevens and Mr. Wakefield exaggerated trifling cases in order

to enhance the value of their efforts."79 While the editors do not immediately indict the reputation of Stevens, they do urge him to call for the government to appoint a commission to investigate the treatment. The change in tone is due to reports in other journals that Dr. Stevens' treatment was only used on prisoners exhibiting premonitory symptoms; this is substantiated a few weeks later by a letter from Sir David Barry observing that he had seen no cases of cholera in Coldbath-Field Prison.80 In 1832, "premonitory diarrhea" was not viewed as a part of cholera. Only "rice water evacuations," as opposed to fecal diarrhea, constituted the disease. This view would shift in later outbreaks as treating premonitory diarrhea became viewed as the most effective way of halting cholera. Stevens responded to Barry by arguing that there were no cases of cholera collapse in the prison as his treatment prevented people from reaching the state of collapse (though 12 people still died of cholera during the month of July).81 However, the Lancet editors ended up concluding that "the Coldbath-Fields Prison epidemic has been most reprehensibly exaggerated by Dr. Stevens and the magistrates" and withdrew their call for further investigation into the saline treatment.82 This incident provides an early example of the Lancet's desire for large-scale, governmental investigations of medical treatments. The Lancet was weary of individual medical practitioners exaggerating the

efficacy of their treatments to garner attention or merely through their implicit biases. While they do not accuse Stevens of doing so, they intone that other members of the medical profession would shunt him as a "quack" if his results were not replicable. It likely did not help that Stevens had published his findings in the Times. Furthermore, investigation by a government commission would provide governmental endorsement to the saline plan if adopted, a factor which would legitimize the treatment in the eyes of the public, as the government was still likely more trustworthy than the medical profession.

Latta's intravenous version of the saline treatment was also not without controversy. Dr. Craigie, another proponent of saline injections, published a piece of correspondence related to this treatment in the Courant, an Edinburgh public paper, in which he portrayed himself as the sole originator of this practice. In response to this, Latta wrote a letter to the Lancet, railing against the practice of publishing on medical subjects in public newspapers.83 In his letter, Latta states that practitioners writing about medical subjects in newspapers is "a crime for which they have no excuse" and that "no respectable practitioner can possibly be guilty of such empiricism, for, certainly, quackery it is, in the humblest sense of that degrading term."84 Given the context, it is likely that Latta used the term "empiricism" as a synonym

for quackery, rather than to refer specifically to the practice of medicine based on personal experience and observation. Latta believed the practitioners publishing outside of the medical sphere primed them for self aggrandizement as they vied for potential patronage from readers of public papers. This was an issue to be taken very seriously in the medical marketplace. In this particular instance, Mr. Mitchell, a friend of Latta's wrote a letter to the Edinburgh Observer, calling Craigie out for stealing credit. After this "an interview took place, casually, in the street, when a trifling assault with a child's whip was believed by Dr. Craigie to have been committed on him by Mr. Mitchell."85

3. Castor Oil Controversy

Between the 1832 and 1854 cholera epidemics, the 1848-49 cholera epidemic occurred in England. This epidemic was the deadliest wave of cholera to sweep England and spurred the passage of the 1848 Public Health Act, which created the first permanent, centralized General Board of Health tasked with advising the public on sanitary matters and disease prevention and administering local boards of health.86 This institution was headed by Edwin Chadwick from its inception until 1854, when it was dissolved and re-formed under Benjamin Hall. Chadwick and his successors in the General Board had backgrounds as civil administrators, not medical practitioners. Christopher Hamlin has argued that Chadwick's

leading role in sanitary efforts during the 1830s-50s shaped the evolution of public health as a field. While many medical practitioners in the early nineteenth century identified issues such as poor diet and living conditions as threats to health, Chadwick adopted an approach to public health that centered on external disease eradication through modern sewers, constant water supply, and cleaning. In turn, Victorian public health measures after the 1832 epidemic placed greater focus on technical and civil engineering issues than social problems or addressing poverty and its effects.87

While changes were afoot in the realm of public health, little changed in terms of cholera treatments by 1854. During this outbreak, the Times received correspondence on a variety of cholera treatments that tended to echo treatments used in previous epidemics. For example, one surgeon wrote in suggesting a course of treatment including small, frequently repeated doses of calomel, a mixture of rhubarb, ammonia and peppermint water, and half pint draughts of salt and water.88 This prescription demonstrates the eclectic courses of treatment described by most medical practitioners who did not subscribe exclusively to one school of treatment but rather borrowed from modes of treatment advocated by individuals like Dr. Stevens and Dr. Ayre. Medical correspondents in the Times also generally did not publish the pathological theory behind their treatments, merely aiming to publicize

therapies that had proven effective in their personal experience. Several practitioners also wrote the newspaper expressing "the danger of giving by your publicity weightly prescriptions from non-medical men" specifically in reference to excessive doses of laudanum.89 This shows that practitioners believed the Times carried a burden to censor treatments that had been deemed dangerous by the profession at large. The Times sometimes did this, as in the case of laudanum and also in the case of a "Angostura bitters" which was itself safe but contained components that were often substituted with strychnia bark.90

The discourse surrounding cholera therapies would be disrupted by the emergence of a relatively novel treatment into the public eye: George Johnson's castor oil. The treatment was first mentioned in the Times on September 6th in a piece describing Johnson's hypothesis that purging in cholera was the body's effort to expel the poisonous matter causing the disease and that, therefore, the purging ought to be aided using evaculants like castor oil. Castor oil was an "eliminative" treatment aimed at eliminating poison from the body, rather than an "astringent" aimed at reducing diarrhea. The letter adds, "what method of treatment could be more simple than this?"91 The correspondent cites the previous week's Lancet for this information, however, their excitement over the treatment did not reflect the

attitude of the Lancet, which merely stated that most cases treated with castor oil had done well and that it left its readers to judge.92 However, at this point, the excitement over castor oil was probably already growing as the original correspondent to the Times mentions druggists inflating the price of the product in response to increased demand. A few days later, the Times published a piece by George Johnson and editorialized that they were glad at the amount of correspondence they were receiving and that "the rational system of treatment is attracting the attention which it deserves; but we are at the same time sorry to see the jealousy with which the profession at large seem to regard the threatened downfall of the old obstructive system of astringents, opiates, and stimulants."93 The Times viewed existing medical treatments as largely ineffective and challenged medical practitioners to put castor oil to the test instead of blindly dismissing it. It seems that the appeal of castor oil to readers of the Times was that it had simple and clear reasoning behind it: cholera was an external poison, and it had to be expelled from the body. Unlike other therapies in which disease agency was obscured or convoluted, in which symptoms were treated rather than addressing the root cause, Johnson presented a simple story with a simple solution. For members of the public frustrated with decades of ineffective therapies that addressed complex pathological processes,

imagining the "cholera poison" as an invader that need only be removed would have been enticing.

In the following weeks, the Times received letters from many individual practitioners who attested to the efficacy of castor oil in their practices.94 Of these, the only to cite specific statistics was R.H. Bradley, who stated that he attended 26 cholera cases, of which 15 recovered and that all recovered cases and all the cases that recovered were treated with castor oil (he does not state what the other cases were treated with, meaning that castor oil was effective in 58% to 100% of cases).95 Apart from Dr. Ayre, who dismissed Johnson's hypothesis as "wholly untenable,"96 the only voice that spoke against castor oil on the pages of the Times was the surgeon George Sutherland. He conducted a trial of castor oil in eight cholera cases and seven of cases he treated with the remedy died. Sutherland went so far as to include a table featuring the names and ages of the patients, the time and date admitted to the hospital, and the times and date of death.97 This was met with a letter expressing regret at seeing "an attempt to impugn the efficacy of castor oil."98

The General Board of Health soon followed suit in investigating castor oil as a treatment for cholera and conducted a meta-analysis of the reported findings of various practitioners employing the treatment. They found that, out of 89 cases treated by 14 different practitioners, 68 concluded in fatality, 15 recovered, and 6 were still under

treatment.99 In this study, at least 76% of cases treated with castor oil terminated in fatality. The next day, a letter appeared in the paper wondering how the successful results reported in the newspaper could be reconciled with the overwhelmingly negative results of the General Board of Health and intoned that the medical profession closed their minds to "anything that appears contrary to the preconceived notions."100

For the Lancet, the Board of Health's announcement around September 16th that it would begin collecting a systematic record of cholera cases, as well as their treatments and results, beckoned in a new era in which treatments could be assessed on a large scale with the hope of finding the most effective treatment for cholera. With this came the acknowledgement that all current treatments of the disease, "howsoever loudly some of them may be vaunted as rational, are, in truth, empirical, experimental, and tentative." People were prone to rush into theories "according to accidents of observation and accidents of hobbyism." Every new theorist felt compelled to advertise their ideas in "epistolary quackery" and George Johnson was but another such individual.101 According to the Lancet, he asked the profession to adhere as blindly to his system of treatment as he accused them of adhering to others. Johnson also asked his readers to assume a pathology from his authority alone, with his treatment being a corollary of this pathology. The Lancet cautioned the public to be wary of such "posts and propters," the profession already being aware of their real value, and stated that "The condemnation of castor oil and the system of purging, by the profession, is almost universal."102 This statement would have come as a surprise

to anyone reading the Times alone, where the vast majority of medical correspondents sang the praise of castor oil. By describing a consensus against castor oil, the Lancet was again delineating medical discourse in professional journals from the puffery of the public press. It was also separating experiential, empirical medicine from medicine based on large-scale, systematic approaches like the meta-analysis conducted by the Board. Despite the fact that the majority of the Lancet's correspondence was based on the experiences of individual practitioners, the editors placed greater credence in the surveys conducted by the Board of Health that focused on the replicability of results among practitioners.

The next week, the Lancet took an even more forceful stand based on reports that hundreds of people put off seeking medical treatment and instead placed their faith in castor oil, often terminating in fatality. The editors stated that the "history of this delusion offers a memorable example of the evil of medical prescribing in the newspapers."[103] Medical practitioners who published "certain cures" in the public press lost sight of professional decorum and duty to the public, their behavior only explained by "the supposition that they think medical science is likely to be advanced by public discussion in the newspapers. This is tantamount to advocating universal suffrage in Medicine."[104] This is perhaps the most strident stand the Lancet took on medical publishing in relation to cholera. The editors believed that discussing medical science in public papers allowed every unqualified individual to have

an equal say in a field in which a bedrock of specialized knowledge and training was required to participate. Medical discussion should, instead, be restricted to the pages of professional journals, after which a consensus finding could, presumably, be transmitted to the public. If this discussion initially occurred in the public sphere, people illiterate in the field of medicine could be misled by untested treatments and, moreover, the authority and exclusive knowledge base the medical profession was attempting to establish would be challenged by pluralistic and conflicting opinions.

The Situation by 1866

As the Lancet observed in the 1832 epidemic, times of cholera were characterized by a "turmoil of rashness and excitement" that obscured facts and did not allow for thorough investigations.105 However, in the aftermath of the 1854 epidemic, more thoroughly developed theories about cholera etiology began to gain publicity. The 1854 outbreak in London was unique from its predecessors as it primarily struck the respectable and relatively more affluent neighborhood of St. James. Previous epidemics had been mostly centered in poorer areas in east and south London, contributing to the perception of cholera as a disease of filth and poverty. As illustrated earlier, the appearance of cholera among middle and upper class individuals in the summer of 1832 may have contributed to a softening of the Times' anti-contagionist stance.

Pamela Gilbert has argued that the outbreak of cholera in St. James lent credibility to John Snow's theory that cholera was transmitted through water contaminated by human excrement in 1854. Water had been identified as relating to cholera since the disease first appeared in England and Snow had forwarded his hypothesis as early as 1849. However, it was only the prevalence of cholera in an area defined by "whiteness, lightness, Englishness, and cleanliness" like St. James rather than one of "racial degeneration, darkness [...], Irishness, and filth" that allowed cholera to be disentangled from place, visible filth, and moral degeneracy in the minds of English officials.106

   Apart from the growing acceptance of cholera as a disease transmitted via contaminated water between 1854 and 1866, this period also saw increases in medical professionalization and the integration of the medical profession into the state apparatus. By mid-century general practitioners' struggles for professional reform were reaching their height. After 1840, seventeen bills advocating for various forms of professional regulation were presented before Parliament; the last of these passed as the 1858 Medical Act. This act created a national register held by the General Medical Council of medical practitioners who were licensed by one of the twenty-one licensing bodies in existence at this point. It also extended the licensing authorities of all these bodies to cover

the entirety of the United Kingdom and allowed licensed practitioners to practice in any of the three areas of medicine. The act did not, however, outlaw unlicensed medical practice.107 The passage of the act brought the medical professional under the purview of the government, lending the government's tacit endorsement to medical practitioners.

By 1866, medical practitioners and medical knowledge were beginning to find their place within government as well. In 1858, the General Board of Health was dissolved and its duties were assumed by the Medical Department of the Privy Council, which was led by John Simon as its Medical Officer. John Simon was a surgeon and had worked as the first Medical Officer of the City of London since 1848. In 1855, the appointment of Medical Officers of Health became compulsory for all London's districts, signaling further integration of the profession into the governmental bureaucracy. Simon is credited by Margaret Pelling with "eliminating the gap between [official] doctrine and the opinions of the profession as a whole."108 He aimed to create a scientific basis for sanitation and public health and conducted similar epidemiological investigations to John Snow following the 1854 epidemic as well as repeating experiments on mice by the German surgeon Karl Thiersch that tested the possibility of fecal-oral transmission of cholera. Combined, these

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investigations by Simon convinced him that cholera was capable of being transmitted fecal-orally through contaminated water, though he did not believe this was exclusively true.109

The General Register Office also played a key role in the 1866 epidemic. Official registration of causes of death had begun in 1837, largely in response to the 1832 cholera epidemic. From 1838 onwards, William Farr, a trained apothecary, held the position of "Compiler of Abstracts" in the office. In this position, Farr developed methods for predicting the rise and fall of epidemics and conducted studies into the epidemiology of cholera, the first of which centered on the 1848-49 epidemic. In this, he identified "cholera fields" where the disease hit hardest and found correlations between income and elevation in relation to cholera mortality. While this could be linked with miasma theory, Farr also entertained Snow's ideas and noticed that the cholera fields also correlated with areas serviced by specific water companies. He tested this again during the 1854 outbreak and came to the conclusion that "cholera matter" was largely diffused via water.110

In short, by the time cholera reemerged in 1866, the medical profession was becoming increasingly integrated into the British government and medical professionals occupied high-level, highly public positions within the bureaucracy. The Times published the first reports of the General Register

Office, including discussions from Farr, weekly, and gave prime billing to statements from the Medical Officer of the Privy Council. Due to how the 1854 cholera outbreak upset conventional thinking on the disease and their own scientific studies, the public mouthpieces of these two offices were in agreement that cholera was often transmitted through water contaminated by human excrement. This was the first time that this type of agreement existed among highly visible medical practitioners who were endorsed by the government going into an outbreak, a set of circumstances which would shape how discourse surrounding the epidemic evolved.

Cholera Discourse in 1866

In previous epidemics, the Lancet had decried the old Board of Health for its lack of representation of medical professionals,111 and attributed the lack of authority of the profession to the fact that "they do not hold high and commanding lucrative offices of state."112 However, in 1866, it took pleasure in the integration of the medical profession and the government, commenting that the Office of the General Register was closely connected with the profession and that the Registrar's main collaborator (Farr) "is one whose sympathies would naturally be with a body of which he himself is an esteemed member." They add that the recommendations of Dr. Farr would be shared by the journal.113 The Times also endorsed the views

of these offices. The newspaper had published reports from the Registrar General every Wednesday under the heading of "Public Health" since the commencement of registration and had printed recommendations from the General Board of Health when it was in operation. The appearance of this governmental medical knowledge was not new to the Times. However, it would take on a new significance in 1866.

In July of 1866, the Times published a report from Dr. Simon to the Privy Council stating that a person traveling from a place under epidemic influence may convey the infection to other locations through discharges that underwent decomposition. Furthermore, discharges from a cholera patient could seep into drinking water supplies, with the potential to infect a large population.114 Three days later, the Times editorial reiterated these views, saying that Simon's statement "give distinct account, on the highest authority, of the principle facts ascertained respecting the nature and contagiousness of Cholera."115 The Times ascribing "highest authority" to the Privy Council Medical Officer is a far cry from 1832, when the newspaper gave anonymous correspondents about the same degree of authority in swaying its editorial opinions. This is indicative of both the standardization of medical knowledge through the filter of governmental officials and the Times' turn away from pluralistic knowledge generation in the

later part of the nineteenth century. Having one medical opinion echoed throughout the government rather than a cacophony of discordant ones from private practitioners lent great credibility to official medical knowledge. Furthermore, as discussed previously, the Times relied on a loose network of correspondents for virtually all its information in the 1830s as these could often ensure faster apprehension and dissemination of knowledge. However, by the 1860s, governmental dispatches were becoming faster and more reliable, surpassing unofficial correspondent networks.116

Just because the Times' editors agreed with governmental medical authorities on the transmission of cholera did not mean that all its correspondents were in accord or that old theories were completely gone. For example, some correspondents, including those with medical credentials, complained of public nuisances or other hazards cited foul airs as risks for cholera.117 The connection between cholera and atmospheric conditions also continued to prevail, with the meteorologist James Glaisher writing to the paper to report an unmovable blue mist that he observed hanging over London which had also been present during previous cholera outbreaks.118 A few correspondents wrote to confirm the existence of this mist, with one connecting it with the presence of a comet.119 Despite the coexistence of

atmosphere related beliefs in 1866, these beliefs did not come into direct conflict with the idea of water-based transmission in the pages of The Times. It is therefore possible that individuals expressing such observations were either not informed on the new developments in cholera etiology or that they simply merged these into their pre-existing views on disease. This demonstrates the proliferation of contingent-contagionist thought in the 1860s, as even Simon and Farr did not believe that cholera was exclusively transmitted from person to person through water.

While the Privy Council's reports informed the public of the method of cholera transmission, information regarding the distribution and the root cause of the 1866 outbreak came from the Office of the General Register. Based on the developments in thought in the aftermath of 1854, reports from the office in 1866 were far more conscious of the geography of cholera than ever before and, beyond that, made inferences based on the disease's geographic distribution. At the beginning of August, the Registrar's report in the Times stated that the areas affected by cholera all derived their water from the East London Waterworks Company, which drew its water supply from the Lea River. The report went on to argue that filtration did not guarantee the purity of water and that London's water supply needed reform, beginning with the abolition of tanks and butts and their replacement

with a constant water supply. The report concluded that local authorities had been largely paralyzed throughout the outbreak and that "The people of East London want help."120 Unlike in previous epidemics, the Registrar was not only reporting statistics and geographic distribution of disease, but investigating the causes connecting cholera cases and adopting an advocacy stance. The Times editorial reiterated the statements of the Registrar a few days later while also critiquing the claims by the engineer of the East London Waterworks Company, published in correspondence the day before, that water derived from a rural district would necessarily be clean. The editors went on to express happiness at the fact that the Privy Council would be conducting a public inquiry into the epidemic, adding that "Medical men who are in the heat of the epidemic have no time for careful observations and experiments, still less are they able to collect general statistics, and they often do not possess the authority which is requisite for a complete prosecution of their investigations."121 The Times believed investigations by private medical practitioners into the epidemic were not a desirable mode through which to obtain information given their small scale and limited resources. Similar to the Lancet's conclusions about cholera treatments in 1854, the Times decided that only large-scale investigations could produce generalizable results; furthermore, these

investigations should be conducted by medical officials in the government. With some exceptions,122 the time of personal, empirical observations germinating myriad different theories of disease was giving way to a single, officially endorsed narrative of cholera transmission.

Despite the increasing role of the government in investigating and generating knowledge related to cholera, the Lancet still felt that the majority of the burden to research these issues fell on regular medical practitioners, who also had to spend their time treating individual cases on top of recording and trying to make sense of all the circumstances surrounding them.123 Underlying this statement is the Lancet's enduring frustration with the empiricism prevalent in cholera literature. Practitioners in the midst of treating cholera were necessarily so involved in their cases that these would shape their opinions and judgements, leading to a mass of medical literature that was merely an assortment of disparate and often conflicting personal experiences. What the Lancet believed was needed instead was a commission that, unclouded by the biases of personal experience, could reach generalizable conclusions. The journal called for the appointment of a government commission to fulfill these duties and when this did not happen, it decided to form its own commission to investigate to what extent the water supply caused the spread of cholera.124 The

formation of "Lancet Sanitary Commission on the Epidemic of Cholera in the East End of London" came right on the tail of the "Lancet Sanitary Commission for Investigating the State of the Infirmaries of Workhouses," whose work dominated the public press earlier in the summer. The Commission was followed by an investigation by H.B. Farnall, the Commissioner of the Poor Law Board, who was described by the Lancet as being appointed to follow in the footsteps of their commission.125 By establishing commissions like these, the Lancet was taking an active role in generating the type of public health information it asked for. The journal also found that the government was receptive to its investigations, starting their own based on those of the Lancet. Through this, the journal could nudge the government into conducting investigations into public health and create the broad, large-scale data for which it had been advocating.

Apart from itself taking steps to counter empiricism in public health, the Lancet also called on the medical profession at large to do the same. The 1866 Sanitary Act made the removal of nuisances and improvement of sanitary conditions compulsory for local authorities, creating stricter standards than the Public Health Act of 1848. With the passage of this act, the Lancet called the medical profession into action, urging "our professional brethren" to "avail themselves at once

of the opportunity now afforded them of assuming their rightful place as conservators of the public health.".126 This phrasing is interesting as the abilities endowed to medical practitioners under the Act were the same ones given to the public as a whole, namely the ability to appeal to the government to compel local authorities to make sanitary changes. However, the Lancet attributed special power to the medical profession through their shared understanding of sanitary science and their ability to unite as an influential group. They state that "the united application of respectable practitioners of a town or district" would have a powerful influence on sanitary authorities, even more so with the backing of the Home Secretary.127 Therefore, the Lancet implored "our brethren to unite in one great effort to raise the tone of public spirit throughout the profession to such a level as shall render it impossible for any medical men, with the slightest regard for his reputation, to offer factious assistance to the enemies of sanitary improvement."128 Once again, the Lancet called for the medical profession to unite and warned against the threats of pluralism in issues of public interest. They were afraid of medical practitioners being swayed by the state of their employment to ignore sanitary threats. Because of this, they called for the formation of sanitary associations among practitioners so that they could act as a united front. Similar to how the

Lancet had attempted to restrict medical discussion from the pages of public newspapers so that the profession could present a united front of knowledge in terms of treatment, they also advocated for practitioners to reach agreements about sanitary issues amongst themselves before petitioning publicly to give the impression of uniformity of medical opinion. The Lancet was aware of how a plurality of medical opinions could be used by public officials or publications to equivocate and push their own agendas. The journal's appeals for the abandonment of empiricism were not confined to public health; in fact, they were most plaintive in regard to treatment.

Despite the seemingly conclusory reports from the Board of Health in 1854, the craze over "eliminative" treatments for cholera continued on the pages of the Times in 1866. By this time, most medical practitioners recognized treating the "premonitory diarrhea" as the most effective means of arresting cholera and this idea was also supported by the Privy Council .129 However, the question remained as to what the best way to treat these symptoms was, and, more importantly, how to treat cholera once it progressed past premonitory symptoms. The Times, once again, decided to endorse the work of George Johnson, printing excerpts from his pamphlet which now focused specifically on the "bilious diarrhea" that

marks the onset of the disease. The Times comments on these excerpts that "The acknowledged failure of the repressive system, [...] renders it at least as possible that the opposite system may be both theoretically and practically sound" and argues that Johnson's treatment deserves a trial.130 The fact that the Times was still advocating for a trial of Johnson's treatment after the 1854 debacle implies that they held the General Board of Health's findings as inconclusive and were unfazed by the Lancet's chiding. While the medical profession may have reached general consensus about cholera's mode of transmission and means for preventing it, the same could not be said for treatment. As a result, the newspaper still felt qualified to conjecture about which treatments might be sound.

In the coming weeks, medical practitioners provided the newspaper with a variety of treatments; some endorsed the eliminative treatment and favored purgatives,131 while several advocated variations of the astringent treatment.132 One advocate of the astringent treatment was Waller Lewis, the medical officer for the post office, who supplied postmen with opium confections to be taken with the first appearance of diarrhea.133 Lewis' remarks provided the foundation for a heated debate that raged for about a week in mid-August in which advocates of the eliminative and astringent treatments argued about what exactly the stance of the College of Physicians was on the use of laxatives.134 The debate culminated in a letter from Johnson himself in which he stated that "the time has come when those who put forth their

opinions must be prepared to give reason for them, and defend them before the world."135 Prior to this, nearly all of the parties involved in the debate went out of their way to disclaim that the Times was not an appropriate forum for this type of discussion, yet proceeded to use the public paper as a forum. Johnson, on the other hand, was clearly in favor of the public airing of disagreements between medical practitioners as well as public explanation and justification of therapies that were pursued.

The astringent versus eliminative debate played out quite differently on the pages of the Lancet. At the beginning of June, the Lancet editorial devoted a section to discussing the "peculiarities" of Johnson's theory. It argued that "choleraic collapse" due to "exhaustion" was common and that, therefore, Johnson's method of exciting diarrhea would worsen the severity of collapse. Furthermore, the Lancet pointed out that "there is a discrepancy between Dr. Johnson's experience and that of the profession. His treatment was largely tried in 1854, and generally and strongly disproved."136 In the coming weeks, the Lancet editorial grew increasingly disconsolate about the medical profession's refusal to abandon treatments that had proven ineffective, describing this as "a libel on medicine."137 The editors firmly believed that no therapy presently employed could successfully treat cholera and urged practitioners to rather not administer any treatments than ones that had no effect, as this could at least elucidate the natural progress of cholera.138 Still, every week, its

pages were filled with the specifics of practitioners' therapies that all utilized the same rotating gallery of common cholera treatments, leading the editors to bemoan the "current blind empiricism."139

These editorials demonstrate the Lancet's growing dissatisfaction with empirical treatments, in which every physician based their prescriptive plan on their own observations and experience, rather than large studies and trials. Johnson's beliefs may have been borne out in his experience, but larger bodies of evidence tended to disprove them. However, the same went for virtually all practitioners – Johnson just had the poor decorum of parading himself in public papers. In September, the Lancet published an editorial highlighting the pitfalls of the current method of reporting and developing therapies. It states that conclusions are quickly adopted and that "there is hardly any remedy that can be mentioned for which triumphant success has not been claimed, either on the basis of the collected results of a long series of cases, or the individual experiences of some one practitioner."140 The editorial goes on to compare two sets of data received from Liverpool, one from Dr. M'cloy, an individual physician who claimed that castor oil was highly effective for him, and one from the Liverpool Workhouse Hospital. While M'cloy reported a 34% mortality rate while using castor oil, the Hospital experienced a 50% mortality rate with the same treatment. However, the

Hospital treated more than three times the number of patients with castor oil. This leads the Lancet to conclude that statistics dealing with small figures, like those of M'cloy are of very little value.141 This editorial represents the solution the Lancet posits to the problem of empirical treatments. With the current format of data dissemination, practitioners would continue to report their individual experiences, clouded by individual biases, with no means of assembling all this information into anything larger and more meaningful. However, through large statistical studies of the efficacy of treatments, practitioners would be able to take a step back and view the issue unclouded by personal biases. While the Lancet's goal of creating consensus among the medical profession had largely succeeded in terms of thought on cholera etiology by 1866, the same could net be said for cholera treatments, which remained an area that the journal was desperately trying to mold into a coherent, uniform approach.

Conclusion

Throughout the nineteenth century, the authority of medical knowledge was constantly in contention. In the 1830s-1850s, no consensus existed about the etiology or treatment of cholera and a myriad of opinions were propounded in public and medical publications. At this time, the Times seized upon the discord to implicitly forward an anti-contagionist agenda by endorsing and

providing a platform to medical practitioners who agreed with them. However, the Lancet, convinced that cholera was contagious, decried these practitioners as attention seekers, chasing the "ephemeral puff" of notoriety in the daily press. These sentiments were also shared by members of the medical profession who viewed practitioners who published in newspapers as ruthless self-promoters. In discouraging medical practitioners from publishing in public newspapers, the Lancet was also attempting to carve out separate spheres for medical and public discussion, which would allow medical practitioners to achieve a consensus amongst themselves before exhibiting these findings to the public. The Lancet, in attempts to generate medical consensus, called for large-scale investigations into the efficacy of treatments like saline fluid and castor oil. By 1866, the medical profession had grown increasingly integrated into government bureaucracy, which both served to medicalize public health and legitimize practitioners. Through the mouthpieces of the growing medical bureaucracy, an official, government endorsed consensus began to emerge regarding the etiology of cholera. This was readily accepted by the Times and praised by the Lancet. However, disagreement about cholera treatment persisted amongst medical professionals, much to the chagrin of the Lancet, which continued to advocate for large, governmental inquiries into

treatments. Despite a significant amount of change over the century, some of the Lancet's goals continued to evade it.

Issues of medical consensus, spheres of discussion, and the authority of medical knowledge are by no means confined to the nineteenth century. Cholera, upon entering Britain in 1832, was met with many of the characterizations that accompanied the beginning of the COVID-19 pandemic in 2020: denial, incredulity, and conspiracy. However, the circumstances defining both events are very different: in 1832, there was virtually no regulation of the medical profession; most treatments were highly ineffective; no medical consensus existed as to the disease's infectiousness, let alone its fundamental cause; and the government played a minimal role in public health. Given these circumstances, it is relatively easy to understand an anti-contagionist's skepticism or a castor oil advocate's hope in a novel therapy. Similar analysis of the circumstances surrounding current medical controversies could shed light on why people respond in the ways they do and inform new approaches to medical communication, journalism, and public health.

Bibliography
Primary Sources:
The Times
The Lancet

Secondary Sources:

Brown, Michael. "'Bats, Rats and Barristers': The Lancet, Libel and the Radical Stylistics of Early Nineteenth-century English Medicine." Social History (London), 39, no. 2 (2014): 182-209.

Eyler, John M. Victorian Social Medicine: The Ideas and Methods of William Farr. Baltimore: Johns Hopkins University Press, 1979.

Gilbert, Pamela K. "'Scarcely to Be Described': Urban Extremes as Real Spaces and Mythic Places in the London Cholera Epidemic of 1854." Nineteenth Century Studies, 14 (2000): 149-172.

Guerrant, Richard L., et al., "Cholera, Diarrhea, and Oral Rehydration Therapy." Clinical Infectious Diseases, 37, no. 3 (2003): 398-405.

Hamiln, Christopher. Public Health and Social Justice in the Age of Chadwick: Britain 1800-1854. Cambridge: Cambridge University Press, 1998.

Hobbs, Andrew. "The Deleterious Dominance of The Times in Nineteenth-Century Scholarship." Journal of Victorian Culture, 18, no. 4 (2013): 472-497.

Lang, Sean. Parliamentary Reform, 1785-1928. London and New York: Routledge, 1999.

Liddle, Dallas. "The News Machine: Textual Form and Information Function in the London 'Times', 1785–1885." Book History 19, no. 1 (2016): 132-68.

MacGillivray, Neil. "Dr Thomas Latta: the father of intravenous therapy." Journal of Infection Prevention, 10, no. 1 (2009): 3-6.

Markovits, Stefanie. "Rushing Into Print: "Participatory Journalism" During the Crimean War." Victorian Studies 50, no. 4 (2008): 559-586

Morris, R.J. Cholera 183: The Social Response to An Epidemic. London: Croom Helm, 1976.

Parry, Noel and José Parry, The Rise of the Medical Profession. London: Croom Helm, 1976.

Pelling, Margaret. Cholera, Fever, and English Medicine, 1825-1865. Oxford: Oxford University Press, 1978.

Pladek, Brittany. "'A Variety of Tastes': The Lancet in the Early-Nineteenth-Century Periodical Press." Bulletin of the History of Medicine, 85, no. 4 (2011), 560-586.

Porter, Roy. "Before the Fringe: 'Quackery' and the Eighteenth-Century Medical Market." In Studies in the History of Alternative Medicine, edited by Roger Cooter, 1-27. New York: St. Martin's Press, 1998.

Porter, Roy. "The rise of medical journalism in Britain to 1800." in Medical Journals and Medical Knowledge. Edited by William Bynum, Stephen Lock, and Roy Porter, (London: Routledge, 1992), 6-28.

Reader, W.J. Professional Men. New York: Basic Books, 1966.

Zeheter, Michael. Epidemics, Empire, and Environments : Cholera in Madras and Quebec City, 1818-1910. Pittsburgh, University of Pittsburgh Press, 2015.

[1] Sean Lang, *Parliamentary Reform, 1785-1928,* (London and New York: Routledge, 1999), 26-66.

[2] Noel and José Parry, *The Rise of the Medical Profession,* (London: Croom Helm, 1976), 104

[3] W.J. Reader, *Professional Men,* (New York: Basic Books, 1966), 17-18, 33-43

[4] Parry & Parry, 1976, 104-117.

[5] Reader, 1966, 16-17, 62.

[6] Michael Brown, "'Bats, Rats and Barristers': *The Lancet*, libel and the radical stylistics of early nineteenth century English medicine," *Social History,* 39, no. 2 (2014): 182-83, 206

[7] Michael Worboys, *Spreading Germs,* (Cambridge: Cambridge University Press, 2000), 28-29

[8] Margaret Pelling, *Cholera, Fever, and English Medicine, 1825-1865* (Oxford, Oxford University Press, 1978), 101-104

[9] Worboys, 35-37.

[10] Pelling, 76-79.

[11] *Lancet*, 14 July, 1866, p. 44.

[12] Andrew Hobbs, "The Deleterious Dominance of *The Times* in Nineteenth-Century Scholarship," *Journal of Victorian Culture*, 18, no. 4 (2013), 473-476.

[13] Brittany Pladek, "'A Variety of Tastes': The *Lancet* in the Early-Nineteenth-Century Periodical Press," *Bulletin of the History of Medicine,* 85, no. 4 (2011), 569.

[14] Brown, 188-192.

[15] Roy Porter, "The rise of medical journalism in Britain to 1800," in *Medical Journals and Medical Knowledge,* ed. William Bynum, Stephen Lock, and Roy Porter, (London: Routledge, 1992), 12.

[16] Porter*,* 13.

[17] *Times,* 10 July, 1866, p. 8.

[18] *Times,* 12 May, 1854, p. 6.

[19] Stefanie Markovits, "Rushing Into Print: "Participatory Journalism" During the Crimean War," Victorian Studies 50, no. 4 (2008): 561.

[2] Richard L. Guerrant, et al., "Cholera, Diarrhea, and Oral Rehydration Therapy," *Clinical Infectious Diseases,* 37, no. 3 (2003): 398-405.

[22] *Lancet,* 17 March, 1832, p. 865.

[23] *Lancet,* 23 September, 1854, p. 261

[24] R.J. Morris, *Cholera 1832,* (London: Croom Helm, 1976), 21-32.

[25] *Times*, 14 February, 1832, p. 2.

[26] *Times*, 14 February, 1832, p. 3.

[27] *Ibid*, p. 2.

[28] *Ibid,* p. 3.

[29] *Times*, 15 February, 1832, p. 2-3.

[30] *Times*, 16 February, 1832, p. 4.

[31] *Times*, 16 February, 1832, p. 4.

[32] Morris, 97.

[33] *Times*, 18 February, 1832, p. 3.

[34] *Times*, 20 February, 1832, p. 2.

[35] *Times*, 21 February, 1832, p. 4.

[36] *Times*, 15 March, 1832, p. 1.

[37] *Times*, 17 February, 1832, p.3; 20 February, 1832, p. 3; 15 March, 1832, p. 3; (for example)

[38] *Times*, 15 February, 1832, p. 3; 20 February, 1832, p. 3; 23 February, 1832, p. 4.

[39] *Times*, 29 February, 1832, p. 1.

[40] *Times*, 20 February, 1832, p. 3; 23 February, 1832, p. 3.

[41] *Times*, 26 March, 1832, p. 3. Tweedie, in turn, was accused of trying to stir up alarm over cholera three days later

[42] *Times*, 20 February, 1832, p. 2.

[43] *Times*, 15 February, 1832, p. 3; 7 March, 1832, p. 3.

[44] *Times*, 25 February, 1832, p. 4.

[45] Roy Porter, "Before the Fringe: 'Quackery' and the Eighteenth-Century Medical Market," in *Studies in the History of Alternative Medicine,* ed. Roger Cooter (New York: St. Martin's Press, 1998), 4.

[46] *Lancet,* 4 February, 1832, p. 669-684.

[47] *Lancet,* 18 February, 1832, p. 735.

[48] *Lancet,* 25 February, 1832, p. 775-776.

[49] *Lancet,* 18 February, 1832, p. 739-740

[51] *Lancet,* 25 February, 1832, p. 774.

[52] *Ibid,* p. 776.

[53] *Lancet*, 25 February, 1832, p. 775-778. Many members of the Central Board received no payment and medical inspectors

who were not military officers received seven shillings and sixpence per day plus lodging expenses, a far cry from the twenty guineas a day the *Times* had quoted.

[54] *Ibid*, p. 776.

[55] *Lancet,* 17 March, 1832, p. 861.

[56] *Lancet,* 4 March, 1832, p. 806. (The *Lancet* website dates this issue as March 3)

[57] Porter, "Fringe," x.

[58] *Lancet,* 17 March, 1832, p. 865.

[59] *Lancet,* 17 March, 1832, p. 865.

[60] Porter, "Fringe," 7.

[61] *Lancet,* 25 February, 1832, p. 768

[62] *Lancet,* 25 February, 1832, p. 768

[63] Brown, 189.

[64] *Lancet,* 25 February, 1832, p. 778.

[65] Brown, 192; *Lancet,* 19 May, 1832, p. 209-210; 7 July, 1832, p. 441. In the period surveyed, Johnson published three letters in the Lancet, including one containing his views on cholera contagion and one siding with the *Lancet* about a lawsuit in which it was involved. Johnson and Wakley had a contentious relationship. In 1826, they were involved in a libel suit after Johnson made insinuations about a fire at Wakley's house which resulted in Johnson paying damages to Wakley.

[66] *Lancet,* 25 February, 1832, p. 767-768; 4 March, 1832, p. 795-798; 19 May, 1832, p. 209-210; among others

[67] *Times,* February 23, 1832, p. 3.

[68] *Times,* 17 March, 1832, p. 1; 26 March, 1832, p. 3.

[69] *Times,* 11 July, 1832, p. 2.

[70] *Lancet,* 28 April, 1832, p. 124.

[71] *Lancet,* 4 February, 1832, p. 653-654; 18 February, 1832, p. 744-745.

[72] *Lancet,* 4 February, 1832, p. 652-653.

[73] *Lancet,* 21 April, 1832, p. 94-95.

[74] *Lancet,* 14 April, 1832, p. 43-46

[75] *Lancet,* 26 May, 1832, p. 225-232

[76] *Times,* 4 May, 1832, p. 2.

[77] Richard L. Guerrant, et al., "Cholera, Diarrhea, and Oral Rehydration Therapy," *Clinical Infectious Diseases,* 37, no. 3 (2003): 398-405.

[78] *Lancet,* 2 June, 1832, p. 275.

[79] Neil MacGillivray, "Dr Thomas Latta: the father of intravenous therapy," *Journal of Infection Prevention*, 10, no. 1 (2009): 3-6

[80] *Lancet,* 2 June, 1832, p. 284.

[81] *Lancet,* 23 June, 1832, p. 378.

[82] *Lancet,* 14 July, 1832, p. 455-456.

[83] *Lancet,* 21 July, 1832, p. 492-293.

[84] *Lancet,* 21 July, 1832, p. 503-506.

[85] *Lancet,* 7 July, 1832, p. 428-430.

[86] *Lancet,* 7 July, 1832, p. 428.

[87] *Lancet,* 4 August, 1832, p. 574.

[88] Morris, 159-173

[89] Christopher Hamiln, *Public Health and Social Justice in the Age of Chadwick: Britain 1800-1854,* (Cambridge: Cambridge University Press, 1998), 8-15.

[90] *Times,* 16 August, 1854, p. 9.

[91] *Times,* 17 August, 1854, p. 10.

[92] *Times,* 25 August, 1866, p. 9.

[93] *Times,* 6 September, 1854, p. 4.

[94] *Lancet,* 26 August, p. 178.

[95] *Times,* 9 September, 1854. It was not uncommon for the *Times* to find publications advocating various treatments for cholera and to publish them, but they were generally relegated to the lower portion of the page, without a noticeable heading.

However, this is the second time that the *Times* decided to publicize Johnson's work and to give it noticeable billing within its pages.

[96] *Times,* 12 September, 1854, p. 10; 14 September, 1854, p. 6; 20 September, 1854, p. 8.

[97] *Times,* 20 September, 1854, p. 8.

[98] *Times,* 15 September, p. 9.

[99] *Times,* 18 September, 1854, p. 9.

[100] *Times,* 20 September, 1854, p. 9.

[101] *Times,* 21 September, 1854, p. 7.

[102] *Times,* 22 September, 1854, p. 8.

[103] *Lancet,* 23 September, 1854, p. 261-262.

[11am 04] *Ibid.*

[105] *Lancet,* 30 September, 1854, p. 282.

[106] *Lancet,* 30 September, 1854, p. 282.

[107] *Lancet,* 25 February, 1832, p.778.

[108] Pamela K. Gilbert, "'Scarcely to Be Described': Urban Extremes as Real Spaces and Mythic Places in the London Cholera Epidemic of 1854," *Nineteenth Century Studies,* 14 (2000): 149-172, 151.

[109] Reader, 66-67.

[110] Pelling, 231

[111] *Ibid,* 229-249

[112] John M. Eyler, *Victorian Social Medicine,* (Baltimore: Johns Hopkins University Press, 1979), 111-120.

[113] *Lancet,* 19 August, 1854, p. 151.

[114] *Lancet,* 21 April, 1832, p. 88.

[115] *Lancet,* 21 July,1866, p. 77.

[116] *Times,* 20 July, 1866, p. 5.

[117] *Times,* 23 July, 1866, p. 8.

[118] See discussion in *Times,* 10 July, 1866, p. 8.

[119] *Times,* 6 August, 1866, p. 5; 9 August, p. 6.

[120] *Times,* 7 August, 1866, p. 12.

[121] *Times,* 9 August, 1866, p. 6.

[122] *Times,* 1 August, 1866, p. 11.

[123] *Times,* 3 August, 1866, p. 7.

[124] The 1866 outbreak saw the reemergence of correspondence from an individual going by the pseudonyms of 'Investigator' or 'S.G.O.' This person had written to the newspaper in 1854 to report their personal experiments in identifying a fungal cause for cholera. In 1866, they again wrote to the paper to chime in on a debate about whether boiling water was effective at eliminating cholera matter from water. They investigated this question using similar methods of fungal cultivation. 'Investigator' was, by all indications, an hobbyist investigator and the continued presence of their correspondence in the *Times* indicates that, while knowledge generation was becoming more homogenized, there was still pluralism to be found. See *Times,* 11 September, 1866, p. 9.

[125] *Lancet,* 28 July, 1866, p. 97.

[126] *Lancet,* 11 August, 1866, p. 157-160.

[127] *Lancet,* 21 July, 1866, p. 71.

[128] *Lancet,* 18 August, 1866, p. 187.

[129] *Ibid.*

[130] *Lancet,* 18 August, 1866, p. 187.

[131] *Times,* 25 July, 1866, p. 5.

[132] *Times,* 31 July, 1866, p. 4.

[133] *Times,* 7 August, 1866, p. 12; 11 August, 1866, p. 10.

[134] *Times,* 3 August, 1866, p. 7; 9 August, 1866, p. 6.

[135] *Times,* 9 August 1866, p. 6.

[136] *Times,* 13 August, 1866, p. 12; 14 August, 1866, p. 14; 15 August, 1866, p. 7

[137] *Times,* 17 August, 1866, p. 4.

[138] *Lancet,* 2 June, 1866, p. 609-610.

[139] *Lancet,* 11 August, 1866, p. 153.
[140] *Lancet,* 4 August, 1866, p. 125-126.
[141] *Lancet,* 11 August, 1866, p. 153.
[142] *Lancet,* 1 September, 1866, p. 238
[143] *Ibid.*

About the Author

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52. We Are What We Read: The Problem of Representation on Undergraduate Philosophy Syllabi

Mykie Valenzuela

Faculty Mentor: Carlos Gray Santana (Philosophy, University of Utah)

Academic Philosophy suffers from what has been called a "demographic problem." In 2018,only 1% of full-time philosophy professors in the US were black and women professors totaledjust 17%. Progress in recruiting underrepresented groups has lagged far behind other humanitiesdisciplines, particularly in race and gender. I hypothesized, given that undergraduate syllabicontain texts

predominantly written by white and male philosophers that students fromunderrepresented groups are less likely to major in philosophy. This theory was explored using19 semesters of syllabi records from the University of Utah Department of Philosophy, fromSpring 2011 to Fall 2018. Using the Simpson's Diversity Index, originally a biodiversitymeasure. Based on the identities of authors of assigned readings each syllabus receives a scorethat illustrates how representative it is of the different identities of philosophers and authors. Forexample, if a syllabus only includes authors with the same identity, this would score a 0, while asyllabus that represented each identity equally would score a 1. Simpson's was chosen rather thanpercentage or ratio to represent a more robust representation of identity as it combines both theamount of variation between individuals and the number of identities represented on the syllabus.Identity labels were chosen to match the MAP self-report survey information available to thepublic. Authors that appeared more than once on a syllabus were not counted as being multipleentries of identity for that particular record. Identities were recorded by self-report and historicalrecord. The averaged scores of each semester were used to create a longitudinal comparison withundergraduate demographics in the major of philosophy at the University of Utah. Scores of thegender and race of authors of assigned readings, separately, correlate with the gender and race ofundergraduates in the major. The scores of gender correlated more strongly with demographicsthan that of race. Since the University of Utah has a predominately white student population,further research would contribute to the results found in this study. This novel research studyadds to the literature that supports diversifying the philosophical canon, especially as a benefit tounderrepresented students and the field as a whole.

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SECTION VIII

School of Medicine

53. **The Development and Characterization of Biodegradable Paclitaxel-Conjugates for Alzheimer's Disease (AD) Treatment**

Raghad Al-jassimi

Faculty Mentor: Donna Cross (Radiology and Imaging Sciences, University of Utah)

Abstract

Alzheimer's disease (AD) is a clinical-pathologic

condition that is definitively identified at autopsy and 30%-50% of individuals may develop late onset Alzheimer's Disease (AD) by the age of 85. And although the initial trigger of AD is unknown, the accumulation of amyloid plaques and neurofibrillary tangles has been observed prior to neuronal loss and cognitive decline. However, it is still unclear if amyloid is the cause or an effect of other age-related processes including inflammation, cardiovascular disease, metabolic disorders, etc. In recent years, tau-targeting therapies have been under development, including kinase inhibitors, and tau anti-aggregation molecules. However, many of these strategies have been abandoned due to toxicity and/or ineffectiveness.

Background

Current treatments have focus on amyloid removal, but these have not resulted in measurable cognitive improvements, particularly for late stage of AD. Although these treatments might eventually be beneficial for disease prevention, they are inadequate in the neurodegenerative stage. Treatments studied in our lab have been focused paclitaxel (PTX) and investigating its inability to cross the blood-brain barrier (BBB). PTX has been widely used for treatment of various cancers and has shown promising results in the treatment of AD and TBI, however it does not readily cross the BBB. Conjugation of polymers and peptides to PTX

aimed towards solving the issue of BBB impermeability by increasing blood circulation time and chaperoning PTX across the BBB. As a result, we optimized PTX's therapeutic efficacy using polymer-drug conjugate platform technology, resulting in an effective treatment at significantly lower doses. Overall, our findings suggest that microtubule stabilization via PTX is a promising therapeutic target for both AD and TBI, and that the use of Angiopep-2 peptide and polymer-drug conjugate platform technology could improve its BBB permeability and therapeutic benefits for these disorders.

Methods

Radial Water Maze (RTW) tests were used in this study. To conduct the RTW maze test, mice are placed in the center of the maze and timed for 180s or until they find the escape tube. If a mouse attempts to enter a decoy tube for more than 5s, it is returned to the center of the tub by hand. One the mouse reaches the safety box; it is allowed to remain for 60s and given a treat.

Results

The mice were treated four times with two weeks apart. A two-factor ANOVA revealed a significant difference between groups and a group by days interaction, indicating the benefits of the P-PTX-AP2 conjugate. The Helmert a priori individual comparisons test further confirmed that the P-PTX-AP2-treated group performed significantly better

than the saline-treated group and was not significantly different from the wild-type group. The results suggest that the P-PTX-AP2 conjugate is even more effective than intranasal generic PTX in ameliorating cognitive deficits when administered intravenously.
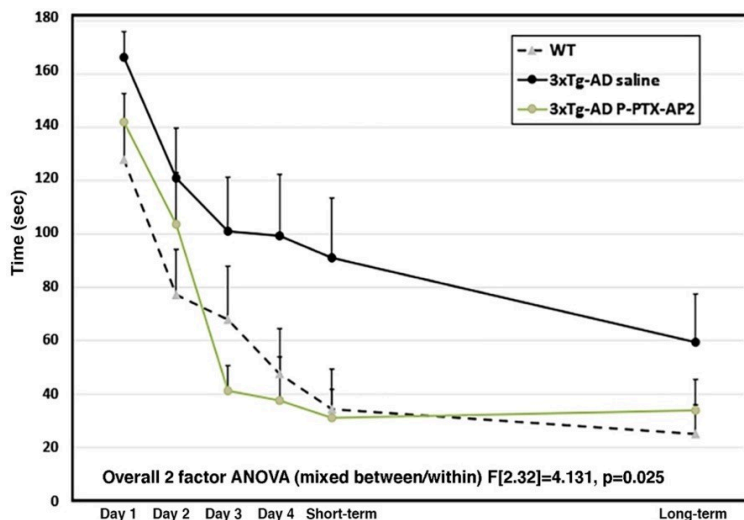
Conclusion

Our study suggests that the P-PTX-AP2 conjugate is effective in ameliorating cognitive deficits in 3xTg-AD mice. This is attributed the AP2 peptide's ability to facilitate brain uptake and the improved biodistribution of the conjugate. Future studies will require PET Imaging to determine pharmacokinetics and a more thorough outcome evaluation. Our research highlights the potential of low-dose paclitaxel as a promising therapeutic approach for neurodegenerative disorders,

particularly Alzheimer's disease. Furthermore, Alzheimer's disease remains a significant global health challenge with no available cure. However, our team's research on the use of low-dose paclitaxel, a microtubule-stabilizing agent, in combination with a brain-targeting peptide, demonstrates potential positive outcomes in preclinical AD models. The novel PTX-conjugates not only improve biodistribution and brain uptake but also exhibit greater efficacy in ameliorating cognitive decline than intranasal generic PTX. Our RWT results showed significat improvement in cognitive ability when comparing treatment stages. This project has high translational potential and could significantly impact the treatment of AD and other CNS conditions such as traumatic brain injury. The availability of a treatment option for these conditions would reduce the enormous suffering experienced by patients and their families and provide a better quality of life for those affected.

Reference

1. Jack CR, etal (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alz Dement 14, 535-562.

2. Congdon, Erin E, and Einar M Sigurdsson. "Tau-targeting therapies for Alzheimer disease." Nature reviews. Neurology vol. 14,7 (2018): 399-415. doi:10.1038/s41582-018-0013-z

3. Cross DJ, Cook DG et al (2021) Intranasal Paclitaxel

Alters Alzheimer's Disease Phenotypic Features in 3xTg-ADMice. J Alz Dis 83, 379-394

Cross DJ, Meabon JS, Cline MM, Richards TL, Stump AJ, Cross CG, Minoshima S, Banks WA, Cook DG (2019) Paclitaxel Reduces Brain Injury from Repeated Head Trauma in Mice. Journal of Alzheimer's disease JAD, 2019. 67(3): p. 859-874.

4. Demeule M, et al, Identification and design of new peptides as a drug delivery system for the brain. J. Pharmacol. Exp. Ther. 324 (2008) 1064–1072.

5. Mei L, et al, Angiopep-2 and activatable cell penetrating peptide dual modified nanoparticles for enhanced tumor targeting and penetrating. Int. J. Pharmaceutics 474 (2014) 95-102.

6. Kopeček J etal HPMA copolymers: Origins, early developments, present, and future Adv. Drug Delivery Rev. 62 (2010) 122-149. PMC2836498.

7. Minko T, Kopeček J, et al Efficacy of chemotherapeutic action of HPMA copolymer-bound Doxorubicin in a solid tumor model of ovarian carcinoma. Int. J. Cancer 86 (2000) 108-117

8. Pan H, Yang J, Kopeček J, etal Polymer-drug delivery conjugates and methods of making and using thereof US 9289510 B2 (March 22, 2016).

9. Yang J, Kopeček J, et al Synthesis of biodegradable multiblock copolymers by click coupling of RAFT-generated heterotelechelic polyHPMA conjugates. Reactive Functional Polym. 71 (2011) 294-302.

10. Luo K, etal Biodegradable multiblock N-(2-hydroxypropyl)methacrylamide copolymers via reversible addition-fragmentation chain transfer

polymerization and click chemistry Macromolecules 44 (2011) 2481-2488 PMC3086388.

11. Yang J, Kopeček J, The light at the end of the tunnel – Second generation HPMA conjugates for cancer treatment. Curr. Opin. Colloid Interface Sci. 31 (2017) 30-42. PMC5739067.

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54. Research Reflection by Raghad Al-Jassimi

Raghad Al-jassimi

Faculty Mentor: Donna Cross (Radiology and Imaging Sciences, University of Utah)

Being a part of a team that is looking at Alzheimer's treatments has been tremendously meaningful for me as a student. It's obvious that Alzheimer's disease has a significant impact on patients and their families, thus the need for effective treatments is constantly present. I'm quite inspired to contribute to the research endeavor as a consequence. It can be challenging to work on this project because it involves a lot of testing, analysis, and collaboration with other scholars. Nevertheless, despite the challenges, the opportunity to positively impact the lives of so many people is tremendously fulfilling.

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55. A Descriptive Analysis of Night Eating Behaviors and Circadian Timing

Mohammad Alrayess

Faculty Mentor: Kelly Baron (Family and Preventive Medicine, University of Utah)

Abstract

Objective: Eating during the biological night is associated with poorer metabolic health. The goal of this study is to examine habitual night eating, eating behaviors in a standardized laboratory task and circadian timing in an ongoing study of circadian
rhythms and eating behaviors.

Methods: Data are derived from a larger ongoing study that focuses on sleep, circadian timing and risk factors for type

2 diabetes. Participants completed self-report questionnaires to assess habitual frequency of night eating and actigraphy to measure sleep. Eating behaviors at night were measured using an eating in the absence of hunger task administered at 21:00. Circadian timing was determined by dim light melatonin onset (DLMO). Metabolic assessments included BMI. This study presents descriptive analyses.

Results: Participants seem to consume more calories of sweet foods compared to neutral foods which seems to correspond with previous studies. Additionally, it seems that participants are able to report their night eating behavior, but are uncomfortable labeling themselves as "night eaters".

Conclusion: This descriptive analysis possibly provides a useful insight on how prevalent night eating might be and the possible eating behaviors during a laboratory task. In the future, analysis of the relationship between DLMO and eating behaviors will be examined.

INTRODUCTION

Obesity in America has been increasing at an alarming rate for many years. Studies show that around 41.9% of Americans are considered to be obese with 9.2% of them being severely obese (Fryar et al., 2020). Many complications can arise due to obesity which include heart disease, stroke, type 2 diabetes, and some cancers (Fryar et al., 2020). Current recommendations largely focus on diet and physical activity to manage obesity risk (CDC, 2020). A growing area specifically is studying the timing of eating throughout an individual's day. Recently, research has started to focus on late night eating as there might be a potential physiological mechanism which contributes to an increase risk of obesity.

Night eating is considered to be a delayed timing of an

individual's meal time which is typically their evening meal (Martinez-Lozano et al., 2020). Identifying whether an individual has night eating behaviors is defined differently across studies, however, several studies define night eating as more than 25% of daily caloric intake after one's evening meal (Tholin et al., 2009). Studies show that night eating seems to be fairly prevalent among adults. One study observed that the prevalence among nonobese men and women seem to be around 4.6% and 3.4% respectively compared to an increase in prevalence among obese men and women with numbers around 8.4% and 7.5% (Tholin et al., 2009). Many issues could arise with night eating. Some studies show that night eating is associated with increased hunger and decreased energy expenditure among obese individuals (Vujović et al., 2022). With this, there has been progress with understanding the relationships between night eating and obesity.

Eating at night may increase hedonic eating, or eating for pleasure. One study highlights that the most commonly consumed food type are snacks and sweets in the late evening (Sebastian et al., 2019). With the timing of food consumption and the types of food being eaten, it is currently understood that this phenomenon might be related to disruption of the body's clock, circadian rhythm (Mendoza, 2019).

In addition, eating at night may be related to disruptions of the circadian rhythm. Studies have shown that meal timings play a role with regulating circadian timing (Wehrens et al., 2017). This is crucial because disrupting one's circadian rhythm leads to unwanted health consequences. One possible consequence that was observed is increased insulin resistance when eating during the biological night (Stenvers et al., 2019). Few studies have quantified eating behaviors relative to the internal biological rhythm. In terms of measuring these

timings, the dim light melatonin onset (DLMO) is used as a biomarker for circadian rhythm and this might influence the metabolic impact of late eating.

A limitation of prior research is that we do not understand how eating relative to DLMO affects eating behaviors and risk for obesity/diabetes. Understanding possible different eating behaviors is crucial since excessive caloric intake is a prime driver for weight gain (Romieu et al., 2017). Understanding an eater's experience with their late-night snacks is essential as well due to the idea that positive eating experiences with hedonic eating is possibly correlated with an increase in appetite (Monteleone et al., 2012).

The objective of this study is to test the relationship between eating in the biological night (after DLMO) with hedonic eating behaviors and metabolic health. In this thesis, we will present descriptive analyses related to the night eating and circadian variables. We will also present our next step planned analyses for next steps to evaluate if those who eat more in the biological night will have poorer metabolic health.

METHODS/DESIGN

Participants

This is a secondary analysis of a longitudinal study that evaluated associations between sleep duration, circadian rhythm, and cardiometabolic health among overweight adults (Baron et al., 2023). Participants were recruited through different means such as flyers through the University of Utah campus, advertisements on websites such as Facebook and Reddit, and participant registries. Participants that were eligible for the study included adults aged 18 to 55 years with a BMI around 25.0-34.9 kg/m^2. They must also sleep on average around 10:30 PM – 3:00 AM which was verified by 7 days of wrist actigraphy. Exclusion criteria include: 1) Chance of

having sleep disorders which were assessed with questionnaires and overnight OSA screening; 2) diabetes diagnosis; 3) history of cognitive or neurological disorders; 4) Presence of major psychiatric disorders as well as possible substance abuse measured with screening questionnaires; 5) Serious medical conditions; 6) Inflexible or overnight work schedules; 7) Any substances that may affect melatonin concentrations such as antidepressants; 8) Current smokers; 9) Daily caffeine intake above 300 mg; 10) Pregnant. (Baron et al., 2023). Once the participants were eligible, the participant inclusion criteria for this study includes that the participant must at least complete the day B visit listed under the longitudinal study. From there, the participant was able to answer the questionnaire related to this study as well as be considered for this study.

Procedure

After pre-screening, participants were scheduled for a screening/baseline visit to determine the participant's eligibility for the study and measure baseline values. The visit included informed consent, HbA1c, height, weight and body fat. After the visit, participants were sent home with a one-night Apnea link to screen for OSA and an actigraphy to help confirm their sleeping times. Participants were eligible for study once they met this criterion (Baron et al., 2023).

Participants completed a circadian phase assessment where saliva samples containing melatonin were collected every 30 minutes starting 6.5 hours before the participant's average bedtime. The lights were dimmed 30 minutes before their first saliva sample and it stayed dim until their average bedtime. Average bed times were estimated by using wrist actigraphy. Participants were instructed to refrain from caffeine and alcohol use for 24 hours before the first saliva sample.

Additionally, they were instructed to refrain from nonsteroidal anti-inflammatory drugs for at least 72 hours before saliva collection.

The eating in absence of hunger task was to measure eating habits within the morning at 9:00 AM and evening at 8:00 PM. Participants were asked to fill out hunger and appetite questionnaires before and after each task. First participants consumed a bowl of plain oatmeal until they stated that they were comfortably full. Then, after a 20-minute waiting period, they were presented with a taste test which had different arrays of palatable and neutral foods such as Oreos or Cheerios and each participant was asked to rate each food on taste, enjoyability, and perception. The study team tracked how much was eaten within each task without the participant's knowledge initially. For the purpose of this study, participants who have at least completed the day B protocol will be considered for this study's purposes as this study is only analyzing evening eating habits.

Measures

Demographics

Participants completed a questionnaire regarding demographics including their sex, age, ethnicity, race, employment, income, and marital status.

Circadian Measures

DLMO was assessed for each individual's profile. This was done by assessing the specific clock time where melatonin initially increases above the average three low daytime values and twice the standard deviation of the baseline values set (Baron et al., 2023). Circadian alignment was calculated by using the participant's DLMO duration and comparing it with the participant's average bed time (Baron et al., 2023).

Evening Calorie Consumption

Calorie consumption of each food item was collected. This was calculated by taking the measured amount of food eaten of each food item and converting that into calories by using the nutritional label associated with each food item.

Taste Testing Rating Form

Measuring participant's self-report of their experience, taste, and enjoyability of certain food items during taste test.

Night Eating Questionnaire (NEQ)

A self-report questionnaire given past participant's day B protocol asking them to report on their assessment on habitual night eating. Questionnaires was either administered through email or during their lab visit. Relevant questions were collected from Night Eating Diagnostic Questionnaire (Geliebter, 2017).

Metabolic Measures

A Tanita scale was used to collect the BMI of every participant.

RESULTS

Demographics

Demographics were collected and results are shown in Table 1 for the 61 participants who completed a questionnaire regarding their demographics. Furthermore, metabolic measures were collected during the first visit of the participant. BMI averages were calculated (Table 1).

Participant Demographics (n=61)	
Demographics	**Mean (SD)**
Age (years)	36.66 (10.16)
Metabolic Measures	
BMI	29.44 (3.04)
	Count (%)
Gender	
Female	27 (44.26)
Male	34 (55.74)
Race	
White	53 (86.89)
Asian	3 (4.92)
Black or African American	1 (1.64)
American Indian/Alaskan Native	1 (1.64)
Native Hawaiian or Other Pacific Islander	1 (1.64)
More Than One Race	2 (3.28)
Ethnicity	
Not Hispanic or Latino	58 (95.08)
Hispanic or Latino	3 (4.92)

Dim Light Melatonin Onset

Of the sample set currently, the average DLMO was calculated (9:00 PM) with the standard deviation. The range of the data was also calculated (Table 2).

Participant's DLMO (n=45)	
	DLMO (pg/ml)
Average	21.07579914
Standard Deviation	1.796034353
Range	11.85511502

Eating in Absence of Hunger Task

Participant's average calories consumed per food item offered to them during the task was calculated (Figure 1). Oreos, Reese's Pieces, and Doritos were reported to have the most

calories consumed during the task on average. On average, Cheerios and Saltines were the food items that were consumed the least.
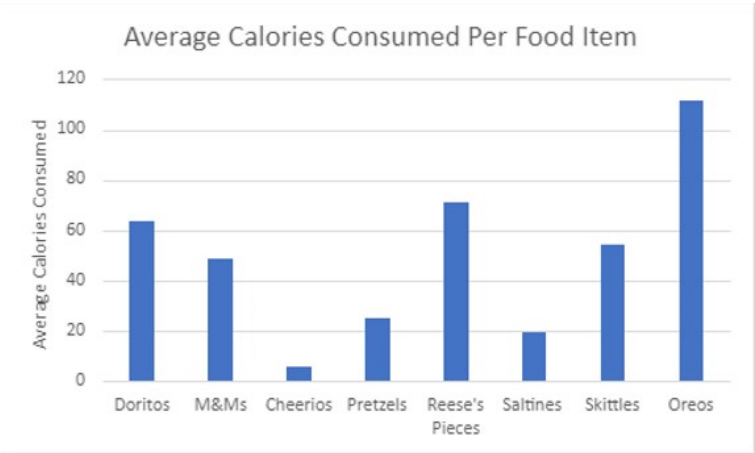
Participants self-reported their experience of how they enjoyed each food item offered. The average ratings (1-100) were calculated from all participants (Figure 2).
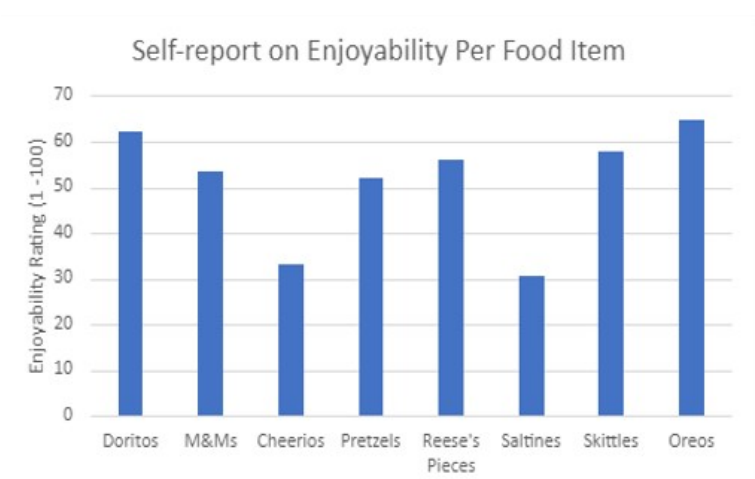
Night Eating Questionnaire (NEQ)

Participants were asked to self-report how much of their daily

caloric intake is after 7:00 PM. Majority reported that 0-10% of their diet comes after 7:00 PM (Figure 3).

How Much of Diet is Consumed After 7:00 PM

- 0 - 10%   - 11% - 20%   - 21% - 30%   - 31%+
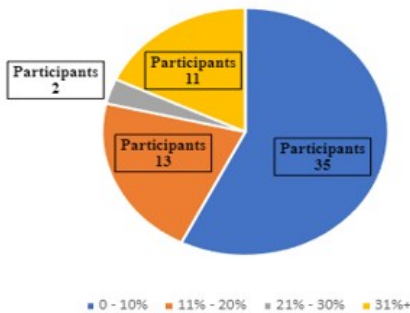
Additionally, participants were asked to self-report their evening meal times. Majority of participants reported their evening meal times to be around 5:30 PM – 6:30 PM (Figure 4).

Participants Self-report Their Times of Their Evening Meals
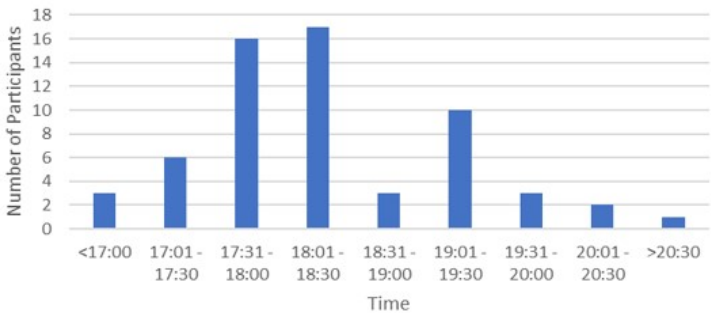
Participants were asked to describe their own night eating behaviors and what would they label themselves as (Table 3).

Participants On Their Night Eating Habits (n=61)

Questions Asked	Count (%)
Q: Most days, do you have urge to eat at night?	
Yes	16 (26.2)
No	45 (73.8)
Q: Would you call yourself a night eater?	
Yes	2 (3.3)
No	59 (96.7)

DISCUSSION

The goal of this descriptive analysis is to examine the prevalence of night eating and eating behaviors in a standardized laboratory task to help further understand these factors and how they might pertain to the overall long-term goal of the study. The long-term goal of this study is to examine evening eating habits compared to individuals' DLMO times. Possible findings could contribute to a better understanding of diseases such as obesity and type II diabetes. Overall, we found that participants consumed a greater number calories of calorie-dense, sweet foods and rated enjoyment higher for these foods as well, even among participants who did not report habitual night eating. Additionally, some participants self-reported to have night eating behaviors (i.e., 26.2% reported having urges to eat at night on most days), but did not label themselves as "night eaters" (3.3%). Finally, participants self-reported their evening meal times with an average of 6:30 PM being calculated and how much of their daily caloric intake is after 7:00 PM with the majority being less than 10%. With this data, there is some analysis that can be done to further understand these data points.

When looking at the average calories consumed, it is intriguing how many of the participants seem to prefer to consume a lot more calories of the sweeter, more calorie-dense

foods compared to the neutral foods offered during the task. At first, this might seem obvious, but when considering the fact that these participants were asked to sample these food items in absence of hunger, one would expect them to eat similar proportions for each food item. However, this does not seem to be the case. Studies have shown that there is evidence of hedonic hunger which is eating for pleasure and that individuals that are considered obese tend to consume a larger portion of high palatability foods compared to normal weight individuals (Lowe & Butryn, 2007). With that, the BMI reported might demonstrate the study's observation since the reported average BMI would be considered to be in the overweight range. This means that these participants might have undergone hedonic eating which could explain the high number of calories consumed for the unhealthy foods even with absence of hunger. More analysis would be needed to observe this relationship.

Additionally, the NEQ results that were collected demonstrated very few participants considered themselves "night eater" (3.3%). This is especially interesting when considering that studies report that younger adults tend to show signs of night eating and it seems to be more apparent as one ages (Striegel-Moore et al., 2006). When considering the average age of the sample was 37 years, out of these participants, one would expect that more of them would self-report as being night eaters. One possible explanation for this observation could be the result of the social desirability bias aspect in this case. When participants were asked to label themselves as night eaters, one might perceive this label to be undesirable. This might be especially true when considering that 26.2% of the participants reported to have some night eating behaviors Reporting their own night eating behaviors

seems to be a relatively comfortable task for the participants to do, but labelling their own behaviors with a term might be making them uncomfortable. More analysis and testing would be needed to identify whether the social desirability bias is a factor in this case.

The participant's evening meal times were recorded and majority seemed to eat their dinner between 5:30 PM – 6:30 PM. Studies show that the average evening meal time is around 6:22 PM (Larson, 2002). This seems to match up with the average evening meal times assessed with the NEQ. Additionally, this seems to be consistent whenever considering how much of the participant's daily caloric intake is after 7:00 PM. Majority of the participants self-reported that 0-10% of their diet is consumed after 7:00 PM. This is consistent with studies that report that 25% of daily calories come from their evening meals (Striegel-Moore et al., 2006). This is relevant as it seems to support the idea that the calories consumed from the evening meals is independent of the excess calories consumed during night eating if it occurs. Understanding this possibility is crucial as it allows for proper analysis of night eating behaviors with these participants. More analysis would be needed such as food diaries to properly understand whether the foods eaten later in the day are associated with commonly eaten foods during night eating.

This study is still on-going. In the next steps, we plan to analyze whether biological timing (DLMO) is associated with hedonic eating behaviors in the eating in absence of hunger task as well as reports of habitual night eating on the NEQ. The calculated average DLMO time was found to be around 9:00 PM. Of these times, 55.6% of the participants had DLMO times below the average and 44.4% of participants had DLMO times above. In the future, this could be analyzed to see whether

these participants tended to consume more calories in general compared to individuals who had a DLMO time before the eating in absence of hunger task.

Our study had several limitations. One limitation are the evening meal times reported could be a case of cultural evening meal times. Depending on the culture, evening meals are consumed at a variety of different times which does not allow for consistency in this aspect. Another limitation is small sample size. Therefore, we may not have the statistical power for comparisons in future analyses. Furthermore, our assessments of eating behaviors were based on self-report habitual intake and a standardized laboratory task. Food diaries were not collected from the participants during this study and may have been a more accurate representation of night eating. Strengths of the study include objective measures of biological timing (DLMO) and measurement of eating behaviors both in the lab and through self-report.

CONCLUSION

This descriptive data analysis possibly provides an insight of night eating behaviors and how individuals might think of their own behaviors. Possible night eating behaviors could have been demonstrated with the observation of increased calorie-intake of sweeter foods among individuals with an average BMI that falls within the overweight range. Furthermore, it seems to be that individuals seem willing to report their own night eating behaviors, however, they feel uncomfortable labeling themselves as "night eaters" which might be associated with a social desirability bias. This is especially true when considering that participants reported times of meals that seem to be consistent with other studies emphasizing that the participants are consistent with their responses The next steps would be to analyze the possible

night eating behaviors in relation to their DLMO time and to possibly further analyze why individuals report their night eating behaviors the way they do.

This study is still on-going; however, the long-term goal is to analyze the relationship between DLMO and eating behaviors in the eating in absence of hunger task. Analysis of this relationship will be able to provide more insight of the data collected and demonstrated within this descriptive data analysis. With this, proper steps can be suggested in trying to aid this issue.

References

Baron, Kelly & Appelhans, Brad & Burgess, Helen & Quinn, Lauretta & Greene, Tom & Allen, Chelsea. (2023). Circadian Timing, Information processing and Metabolism (TIME) study: protocol of a longitudinal study of sleep duration, circadian alignment and cardiometabolic health among overweight adults. BMC endocrine disorders. 23. 26. 10.1186/s12902-023-01272-y.

CDC – National Center for Health Statistics – Healthy Weight, Nutrition, and Physical Activity. https://www.cdc.gov/healthyweight/index.html. June 3, 2022.

Fryar CD, Carroll MD, Afful J. Prevalence of overweight, obesity, and severe obesity among adults aged 20 and over: United States, 1960–1962 through 2017–2018. NCHS Health E-Stats. 2020.

Geliebter, Allan. (2017). Night Eating Diagnostic Questionnaire (NEDQ) Revised (9/2014). 10.13140/RG.2.2.10472.78089.

Larson, Ronald B.. "When Is Dinner." Journal of food distribution research 33 (2002): 38-45.

Lowe, M. R., & Butryn, M. L. (2007). Hedonic hunger: a new dimension of appetite?. Physiology & behavior, 91(4), 432–439.

https://doi.org/10.1016/j.physbeh.2007.04.006

Mendoza J. (2019). Food intake and addictive-like eating behaviors: Time to think about the circadian clock(s). Neuroscience and biobehavioral reviews, 106, 122–132. https://doi.org/10.1016/j.neubiorev.2018.07.003

Monteleone, P., Piscitelli, F., Scognamiglio, P., Monteleone, A. M., Canestrelli, B., Di Marzo, V., & Maj, M. (2012). Hedonic eating is associated with increased peripheral levels of ghrelin and the endocannabinoid 2-arachidonoyl-glycerol in healthy humans: a pilot study. The Journal of clinical endocrinology and metabolism, 97(6), E917–E924. https://doi.org/10.1210/jc.2011-3018

Romieu, I., Dossus, L., Barquera, S., Blottière, H. M., Franks, P. W., Gunter, M., Hwalla, N., Hursting, S. D., Leitzmann, M., Margetts, B., Nishida, C., Potischman, N., Seidell, J., Stepien, M., Wang, Y., Westerterp, K., Winichagoon, P., Wiseman, M., Willett, W. C., & IARC working group on Energy Balance and Obesity (2017). Energy balance and obesity: what are the main drivers?. Cancer causes & control : CCC, 28(3), 247–258. https://doi.org/10.1007/s10552-017-0869-z

Sebastian, R. S., Wilkinson Enns, C., Goldman, J. D., & Moshfegh, A. J. (2019). Late Evening Food and Beverage Consumption by Adults in the U.S. What We Eat in America, NHANES 2013-2016. In FSRG Dietary Data Briefs. United States Department of Agriculture (USDA).

Stenvers, D. J., Scheer, F. A. J. L., Schrauwen, P., la Fleur, S. E., & Kalsbeek, A. (2019). Circadian clocks and insulin resistance. Nature reviews. Endocrinology, 15(2), 75–89. https://doi.org/10.1038/s41574-018-0122-1

Striegel-Moore, R.H., Franko, D.L., Thompson, D., Affenito, S. and Kraemer, H.C. (2006), Night Eating: Prevalence and Demographic Correlates. Obesity, 14: 139-147. https://doi.org/

10.1038/oby.2006.17

Tholin, S., Lindroos, A., Tynelius, P., Akerstedt, T., Stunkard, A. J., Bulik, C. M., & Rasmussen, F. (2009). Prevalence of night eating in obese and nonobese twins. Obesity (Silver Spring, Md.), 17(5), 1050–1055. https://doi.org/10.1038/oby.2008.676

Vujović, N., Piron, M. J., Qian, J., Chellappa, S. L., Nedeltcheva, A., Barr, D., Heng, S. W., Kerlin, K., Srivastav, S., Wang, W., Shoji, B., Garaulet, M., Brady, M. J., & Scheer, F. A. J. L. (2022). Late isocaloric eating increases hunger, decreases energy expenditure, and modifies metabolic pathways in adults with overweight and obesity. Cell metabolism, 34(10), 1486–1498.e7. https://doi.org/10.1016/j.cmet.2022.09.007

Wehrens, S. M. T., Christou, S., Isherwood, C., Middleton, B., Gibbs, M. A., Archer, S. N., Skene, D. J., & Johnston, J. D. (2017). Meal Timing Regulates the Human Circadian System. Current biology : CB, 27(12), 1768–1775.e3. https://doi.org/10.1016/j.cub.2017.04.059

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56. **Cervical Dorsal Root Ganglion Imaging for Studying Neuronal Nociceptive Circuit Changes in Migraine Models**

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Faculty Mentor: K.C. Brennan (Neurology, University of Utah)

ABSTRACT

Migraine neuroscience is a field in its infancy, with migraine

being a remarkably common yet poorly understood sensory circuit disorder. It is characterized by attacks of unilateral, throbbing craniofacial pain, with sensitivity to movement, visual, auditory, and other afferent inputs. Migraines can increase in frequency and intensity over time, transitioning from episodic to chronic migraines that resemble a never-ending migraine attack. The long-lasting, debilitating pain of migraine attacks is thought to follow a circuit of neuronal networks connecting the peripheral nervous system to the central nervous system as it ascends to the cerebral cortex for processing of painful stimuli. Using in vivo two-photon microscopy calcium fluorescence imaging with a novel surgical preparation to image fluorescence from genetically encoded calcium indicators in the C2 dorsal root ganglion (DRG), we establish an imaging paradigm of sensory neurons from the C2 DRG in response to greater occipital nerve and associated dermatome stimulation. We observe that in a nitroglycerin-induced chronic migraine state, a greater number of sensory neurons in the DRG respond when the greater occipital nerve dermatome is electrically stimulated. Additionally, this responder expansion is noted in sodium-nitroprusside-induced acute migraine models. Importantly, the understanding of nociceptive network activation in migraine will allow us to look at changes in the pain circuit that serve as markers for a migraine attack, observe how they occur, and see if they can be reversed at a neuron or circuit level to prevent the pain associated with migraine.

INTRODUCTION

Neuroscience became a distinct discipline of biological science in the late 1950s and early 1960s (Cowan et al., 2000). It has an interdisciplinary scope, combining expertise from physiology, anatomy, biology, cytology, computer science, and

mathematical modeling in order to understand the nervous system. In addition to examining the normal development and activity of the nervous system, neuroscience studies diseases, disorders, and injuries that affect parts of the nervous system, how it develops, and how well it functions (Bear et al., 2016). Systems neuroscience—the study of the function of neural circuits in intact organisms—aims to understand how biological circuits in the brain generate behavior, thought, sensation, and action (Feldman & Scott, 2020). It is an integrative approach that allows deeper investigation into both healthy and abnormal nervous systems (Brennan & Pietrobon, 2018).

Migraine is an extremely common yet poorly understood nervous system disorder that primarily affects the sensory nervous system (Brennan & Pietrobon, 2018). According to the World Health Organization, "Migraine stands as the sixth most common cause of disability on the planet" (Goadsby et al., 2017). It affects 12% of the world's population, and attacks are often incapacitating (Jensen & Stovner, 2008; Lipton et al., 2008). Migraine is characterized by attacks of unilateral, throbbing craniofacial pain, with sensitivity to movement, visual, auditory, and other afferent inputs (Headache Classification Committee of the International Headache Society, 2013). Most migraine attacks feature sensory amplifications: photophobia, phonophobia, osmophobia, and cutaneous allodynia—the perception of light, sound, smell, and normal touch as amplified or painful (Figure 1) (Burstein et al., 2015). Migraine is not simply a headache; the migraine attack is purely the most visible element on a larger continuum of disease.

Figure 1: The migraine attack involves changes in multiple sensory percepts (Brennan & Pietrobon, 2018).

A

allodynia

pain

phono-
phobia

photo-
phobia

nausea

osmophobia
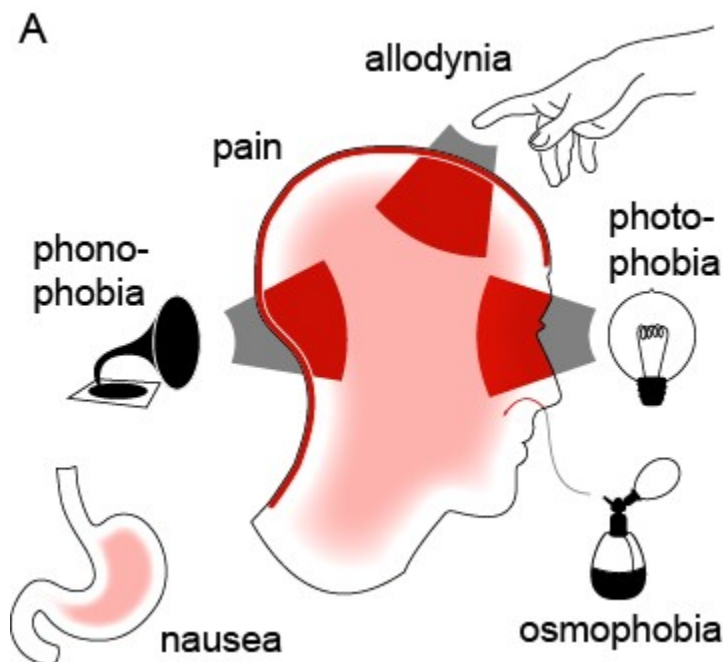
Migraine attacks can increase in frequency over time. Headache experts divide states of progression into no migraine, episodic migraine, and chronic migraine. Chronic migraine is defined by the International Classification of Headache Disorders (ICHD-3) as 15 or more headache days per month. At least eight of those headaches must be migraine attacks, meaning they must be accompanied by one-sided, pulsing pain, increased pain with physical activity, light and/or sound sensitivity, and nausea (International Headache Society, 2013). Chronic migraine affects two percent of the general population, and studies suggest that three percent of people with episodic migraine will transition to chronic migraine each year (May & Schulte, 2016). Longlasting and/or repetitive pain over years leads to profound functional and structural changes in the brain networks (Brennan & Pietrobon, 2018). From an

electrophysiological point of view, chronic migraine resembles a never-ending migraine attack (Coppola & Schoenen, 2012).

The incessant, debilitating pain because of migraine is the most prevalent symptom of all migraineurs. Acute onset and prolonged expression of pain are the leading symptoms that cause individuals to seek migraine treatment through the emergency department. 1.2 million emergency room visits are made in the United States each year seeking medical treatment for migraine (Dodson et al., 2018). In an emergency room situation, physicians prescribe narcotics as the primary treatment option for migraine visits more than 50 percent of the time, despite the fact that there is no specific indication for opiates in migraine treatment (Opioids and Migraine, 2018). In a 2019 study, 19 percent of people with migraine were currently using opioids prescribed by primary care providers specifically to treat migraine—up from the 16 percent reported in 2009 (Ashina et al., 2019). Using opioids to treat migraine has been found to lead to more frequent and severe headaches, as well as rebound headaches; to be a high risk factor in medication overuse headache; to trigger the transition from episodic migraine to chronic migraine; and, when consistently used, to easily become dependent upon and addicted to (Opioids and Migraine, 2018). Most crucially, doctors are treating the symptom of pain rather than the underlying disease of migraine, leading to no real solution.

Furthermore, while other acute medications that treat the potential inflammatory cause of migraine, such as triptans and non-steroidal anti-inflammatory drugs (NSAIDs), exist, a large population of people can't take them, including the elderly, pregnant women, and anyone with a medical history of cardiovascular disease, diabetes, liver cirrhosis, kidney disease, or gastrointestinal bleeds (Opioids and Migraine, 2018). Some

patients do not respond to triptans and other currently available medications. New studies have shown exploration into 5-HT1F receptor blockers and calcitonin gene-related peptide (CGRP) receptor antagonist medication options to treat migraine as they have less cardiovascular risk for patients, but many uncertainties still exist (de Prado & Russo, 2006; Neeb et al., 2010). This means there's a bleak number of viable treatment options to acutely relieve pain and treat migraine that work for everyone, and people deserve to have treatment plans that are effective.

While new data is emerging about the pathophysiology of this debilitating disease (Olesen et al., 2009), little is known about the nociceptive circuit associated with a migraine attack, and in order to truly treat migraine and relieve its most prevailing symptom, the pain circuit must be understood at a deeper level. As a craniofacial pain disorder, migraine is classically considered "trigeminal" in nature, meaning focused within the trigeminal nerve (Brennan & Pietrobon, 2018; Edvinsson et al., 2020). Yet half of the head is covered by the greater and lesser occipital nerves. An emerging consensus is that all sensory nerves covering the head, including the trigeminal and upper cervical networks, can serve as pain substrates and that their effects synergize (Brennan & Pietrobon, 2018). The head pain associated with a migraine attack, including the frontal, temporal, parietal, occipital, and high cervical regions, is thought to be the consequence of activation of the trigeminovascular system and the dorsal root ganglia of cervical roots C1–C3 (Brennan & Pietrobon, 2018; Goadsby et al., 2017). The greater occipital nerve (GON) is the medial branch of the dorsal ramus of the second spinal nerve (C2) (Nielsen, 2020), and clinical GON blocks have shown to have a beneficial effect on chronic migraine preventative

pain treatment (Chowdhury et al., 2021). From an experimental surgical standpoint, the GON circuit offers a critical advantage: it is all accessible—nerve and associated cutaneous territory, dorsal root ganglion, and dorsal horn (Brennan & Pietrobon, 2018). This allows for less invasive surgeries, reduced pain, and decreased potential complications when exploring the migraine pain circuit.

Nociceptive circuitry is the arrangement and functional interconnections of neurons that are responsible for conveying pain information (Todd, 2013). Neurons are differentiated and specialized, and they can be categorized into afferent and efferent neurons based on the function they provide. Afferent or sensory neurons are activated when they receive sensory input from the environment, such as touch, pressure, temperature, light, smell, taste, or pain. They transmit information about changes in homeostasis to the brain. Efferent or motor neurons are employed when the body wants to initiate a response to a stimulus. They send messages from the central nervous system to effector organs that can then carry out actions that restore equilibrium (Bear et al., 2016; Tortora & Nielsen, 2017). A neuron can receive contacts from up to 10,000 presynaptic neurons, and, in turn, a neuron can communicate with up to 10,000 postsynaptic neurons in order to pass information along (Byrne, 1997). The number of possible neuron combinations is what gives rise to complex neuronal circuits and networks that are currently under investigation.

Pain perception circuits begin with free nerve endings, which are branches of the primary neuron. The multitude of different receptors, including nociceptors (pain receptors), convey information that converges onto neuronal cell bodies located in the dorsal root ganglion for stimulus from the body

and the trigeminal ganglia for stimulus from the face (Handler & Ginty, 2021; Kendroud et al., 2021).

Though new genetic markers reveal a multitude of different neuronal types (Handler & Ginty, 2021), there are two major types of nociceptive nerve fibers: A-delta fibers and C-fibers. A-delta fibers are lightly myelinated and have small receptive fields, which allow them to alert the body to the presence of pain. Due to the higher degree of myelination compared to C-fibers, these fibers are responsible for the initial perception of pain. C-fibers, on the other hand, are unmyelinated and have large receptive fields, which allow them to relay pain intensity (Arcilla & Tadi, 2022; Bear et al., 2016; Kendroud et al., 2021).

Nociceptive stimuli activate transient receptor potential (TRP) channels located on nerve endings, which cause first-order neurons to depolarize and fire action potentials. First-order neurons are found in the dorsal root and trigeminal ganglions, which are both considered parts of the peripheral nervous system (Kendroud et al., 2021; Nielsen, 2020; Yam et al., 2018). When receiving pain sensory information from the body, second- order neurons decussate at connections with the central nervous system and then ascend via the lateral spinothalamic tract (Bear et al., 2016; Kendroud et al., 2021; Nielsen, 2020). Third-order neurons are located in the ventral posterolateral nucleus (VPL) and the ventral posterior inferior nucleus (VPI). From the thalamus, nociceptive information projects to the primary somatosensory cortex for further processing and pain perception (Kendroud et al., 2021; Nielsen, 2020). This is the proposed ascending circuit pathway that researchers continue to make inquiries about.

This project focuses on the neural circuits involved in the manifestation of the migraine phenotype, specifically the ascending circuit pathway (Figure 2) that may involve the

cervical dorsal root ganglion (DRG) and central circuits. We aim to understand the nociceptive circuit associated with a migraine attack and the changes that occur in the pain circuit when it becomes a chronic migraine state.

Figure 2: The ascending circuit pathway may be involved in the manifestation of the migraine pain phenotype.
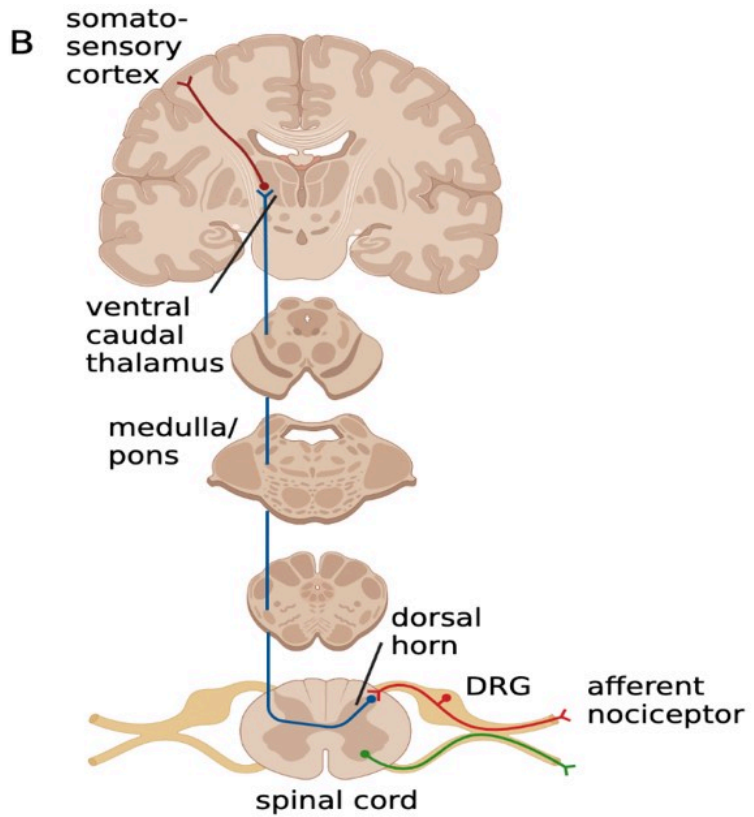
METHODS

All animal procedures are performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Utah as consistent

with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Part 1: Mice Strain

Mice are often used as animal models in neurology because their genetic, biological, and behavioral characteristics closely resemble those of humans in relation to the basic features of pain (Hodge et al., 2019). While non-animal methods of study have made progress in some fields of biomedical research, their use in neuroscience remains extremely limited due to the complex and interconnected structure of the brain. In most cases, a living and behaving organism remains the only viable model to study the brain in action (Jones, 2021).

To selectively study the dorsal root ganglion (DRG), we are using Pirt-1 Cre x Ai95 transgenic mouse lines (Kim et al., 2016). Pirt is a modulator of several transient receptor potential (TRP) channels. Cre recombinase is expressed specifically in Pirt positive neurons, meaning all primary sensory neurons in the DRG and not in any central nervous system neurons (Kim et al., 2008). This allows for direct genetic manipulation and study of DRG neurons. The Ai95 mice supply a genetically encoded calcium indicator, GCaMP6f, that has been widely used for imaging calcium (Ca2+) transients in neuronal somata, dendrites, and synapses of the peripheral nervous system. In theory, this indicator is specific to the peripheral nervous system, which is essential to studying dysfunction of the sympathetic nervous system with regard to migraine (Anderson et al., 2018).

We use both male and female mice that are between 8 and 24 weeks old. This ensures that the mice are mature adults but have not suffered any negative age-related changes, including loss of locomotor activity, sensory and motor function changes,

anxiety-like and depression-related behaviors, and declines in learning and memory.

Part 2: Chronic NTG Migraine Model

Nitroglycerin (NTG) is a vasodilator drug commonly used in the treatment of chest pain and high blood pressure. Since its primary course of action is to dilate blood vessels, an extremely common side effect of this medication is a nitroglycerin-induced headache, though the actual mechanism of how these are related is still unknown (Bektas & Soyuncu, 2010; Sureda-Gibert et al., 2022). In migraineurs, NTG induces a severe delayed headache, resembling a spontaneous migraine attack. Due to this known human migraine trigger, scientists developed a model of chronic migraineassociated pain. Chronic, intermittent administration of NTG to mice has been shown to result in hyperalgesia—an abnormally heightened sensitivity to pain (Moye & Pradhan, 2017).

For purposes of the experiment, half the animals received vehicle (control) injections and half received NTG. Systemic intraperitoneal injections occur every other day for nine days, with five injections total (Figure 3). The NTG is dosed at 10 mg/kg and administered at 10 mL/kg (Moye & Pradhan, 2017). NTG (in 30% alcohol, 30% propylene glycol, American Reagent, NY, USA) is diluted each time in 0.9% saline prior to administration. Vehicle is 30% alcohol, 30% propylene glycol, in saline.

Figure 3: Injections are done on days 1, 3, 5, 7, and 9. Von Frey behavioral testing is performed on days 1, 5, and 9.
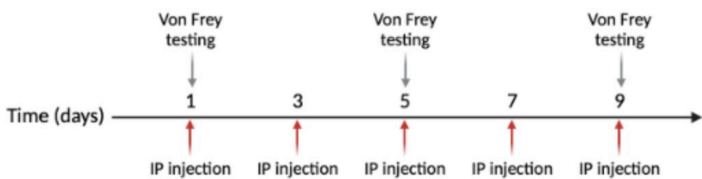
Part 3: Behavior Testing

Acclimation of the animals to the behavior testing room and testing conditions is crucial to reducing confounding variables. This room should be quiet, with low-light conditions, and minimal disruptions during testing to minimize animal stress and variability.

Since pain experience is subjective by nature and cannot be measured directly, pain in animals is inferred based on pain-like behaviors such as withdrawal. Migraine attacks are usually accompanied by sensory amplifications and cutaneous allodynia (a type of pain where touch that usually isn't painful causes severe pain) (Burstein et al., 2015). Manual Von Frey testing is a way to test allodynia in mice via hind paw withdrawal to a pain stimulus. It is completed using monofilaments of differing forces, ranging from 0.008 to 2 grams. Each stimulus is applied perpendicularly to the hind paw. If the rodent withdraws, licks, or shakes the paw, it is considered to have had a positive response to pain. If the animal does not give a response, the next higher gram force filament is used to repeat the procedure and check for a response. If the animal does respond, the procedure is repeated with the next lower gram force filament (Deuis et al., 2017). A basal threshold (baseline) should be completed one hour prior to injections and repeated one-hour post-treatment.

This testing procedure is performed on days one, five, and nine of the experiment to avoid associative learning (Figure 3) (Moye & Pradhan, 2017).

Part 4: Surgery

For less than a decade, surgeries have been performed on mice to view and study the lumbar DRG (Caylor et al., 2019; Chen et al., 2019; Chen et al., 2022). This current study applies similar surgical techniques to the cervical vertebrae to create

a novel procedure that allows in vivo imaging of the C2 DRG (Figure 4).

Figure 4: In vivo two-photon microscopy fluorescence imaging of C2 DRG with greater occipital nerve (GON) stimulation.
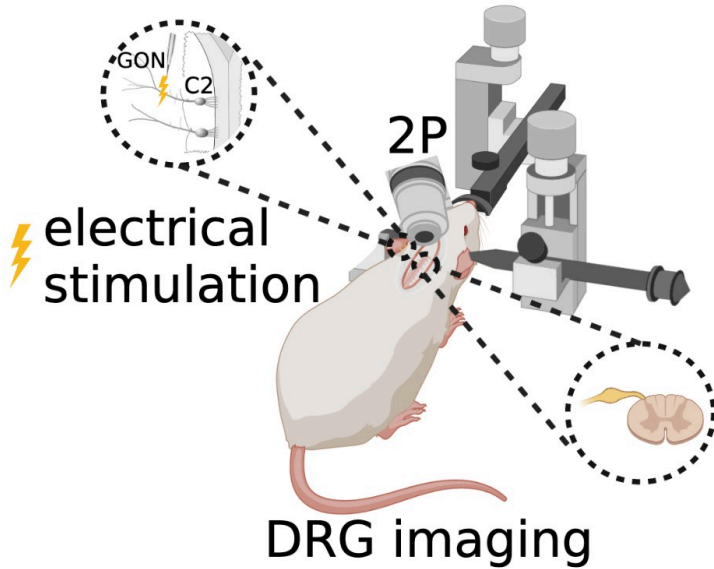
The animal is maintained under deep anesthesia with 1.5–2.5% isoflurane. Anesthesia is verified by the absence of the toe pinch reflex. Ocry-gel is applied to both eyes to maintain eye moisture and prevent corneal abrasions. After shaving the fur at the surgical site and sterilizing the skin with 70% ethanol and 10% povidone-iodine, a small incision is made in the dorsal skin from levels C1– C4 of the spine, and the skin is held back with sutures. Under a stereomicroscope, muscles and Figure 4: In vivo two-photon microscopy fluorescence imaging of C2 DRG with greater occipital nerve (GON) stimulation. ligaments attached to the lateral aspects of the first three vertebrae are detached using surgical scissors. A laminectomy of the C2

vertebra is performed to surgically remove both spinous and transverse processes of the vertebral bone in order to expose the C2 region of the spinal cord and DRG. Once dissected and cleaned away from any remaining fat, meninges, and muscle, the C2 DRG should be clearly visible. A titanium head bar is mounted on the skull in a configuration that allows for stability and clarity of the DRG field of view while imaging.

In vivo two-photon microscopy fluorescence imaging of the genetically encoded calcium indicator GCaMP6f is completed using an immersion objective in an anesthetized state coupled with occipital nerve stimulation.

Part 5: Acute NTG Migraine Model

Retroorbital injections of either NTG (10 mg/kg) or vehicle (6%v/v ethanol, 6%v/v propylene glycol in saline) are given after the mouse is transferred from the surgical station to the imaging platform. A 30-gauge needle connected to a cannula is inserted into the retrobulbar space and positioned to remain in place throughout the course of the imaging. After recording the pretreatment stimulation responses, approximately 100μL of NTG or vehicle solution is injected through the cannula. Post-treatment responses are collected 30 minutes after the injection.

Part 6: Acute SNP Migraine Model

Sodium nitroprusside (SNP), like NTG, is a nitric oxide (NO) donor that has similar effects on sensory behavior (Divakaran & Loscalzo, 2017). Unlike NTG, SNP is water soluble, thus avoiding potential spurious effects from solvents used as vehicle. After the mouse is transferred from the surgical station to the imaging platform and pre-treatment stimulation responses are recorded, a 30-gauge needle connected to a cannula is inserted into the retrobulbar space and positioned to remain in place throughout the rest of the course of imaging.

SNP (2 mg/kg) is dissolved in PBS, and 100μL of SNP solution or PBS vehicle is injected into the retroorbital space. Post-treatment responses are collected at 30- and 60-minutes post-injection. (See experiment scheme below.)
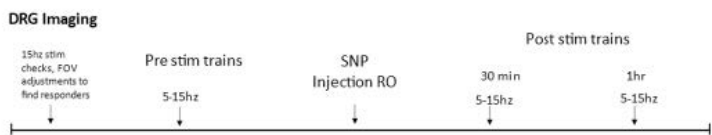
Part 7: Electrical Stimulation

Electrical stimulation is performed with monopolar needle electrodes. The singular electrode is placed against the skin, near the fold between the ear and the neck, to stimulate the greater occipital nerve branching in the C2 dermatome. All pulse parameter paradigms are described below in Figure 5.
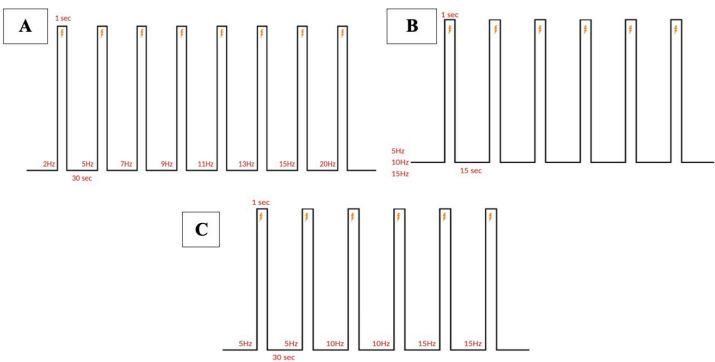
Figure 5: Stimulation paradigm. A. Chronic and acute NTG experiment parameters. 1 ms pulse duration of 0.3 mA at 5, 10, or 15 Hz for a 1 second burst width is repeated six times (at a single frequency) every 15 seconds for one recording. B. Acute SNP experiment parameters. 10 ms pulse duration of 0.3 mA at 5, 10, and 15 Hz for a 1 second burst width, two times at each ascending frequency, every 30 seconds for one recording.

C. Acute NTG experiment parameters. 10 ms pulse duration of 0.3 mA at 2, 5, 7, 8, 11, 13, 15, and 20 Hz for a 1 second burst width, once at each ascending frequency, every 30 seconds for one recording.

Part 8: Data Analysis

Images are recorded from pain-encoding neurons in the DRG, where regions of interest (ROI) of responding cells are determined visually and programmatically (Figure 6A). A critical part of action potentials is the influx of intracellular calcium (Park & Luo, 2010). Calcium entering the neuronal cell throughout these channels is associated with transduction, transmission, processing, and modulation of pain signals (Castro-Junior et al., 2018). The genetically encoded calcium indicator, GCaMP6f, enables recording of the calcium dynamics in the DRG cells in response to the electrical stimulus (Figure 6B). ROI are then converted to raw fluorescence traces in ImageJ using inbuilt and custom-built macros. Raw image stacks are converted to changes in fluorescence Figure 5: Stimulation paradigm. A. Chronic and acute NTG experiment parameters. 1 ms pulse duration of 0.3 mA at 5, 10, or 15 Hz for a 1 second burst width is repeated six times (at a single frequency) every 15 seconds for one recording. B. Acute SNP experiment parameters. 10 ms pulse duration of 0.3 mA at 5, 10, and 15 Hz for a 1 second burst width, two times at each ascending frequency, every 30 seconds for one recording. C. Acute NTG experiment parameters. 10 ms pulse duration of 0.3 mA at 2, 5, 7, 8, 11, 13, 15, and 20 Hz for a 1 second burst width, once at each ascending frequency, every 30 seconds for one recording. A C B ($\Delta F = F_t - F_0$), where F_t is the fluorescence intensity of a given frame and F_0 is the average fluorescence of the first 3-5 seconds. Traces are normalized as $\Delta F/F_0$ and analyzed using Matlab.
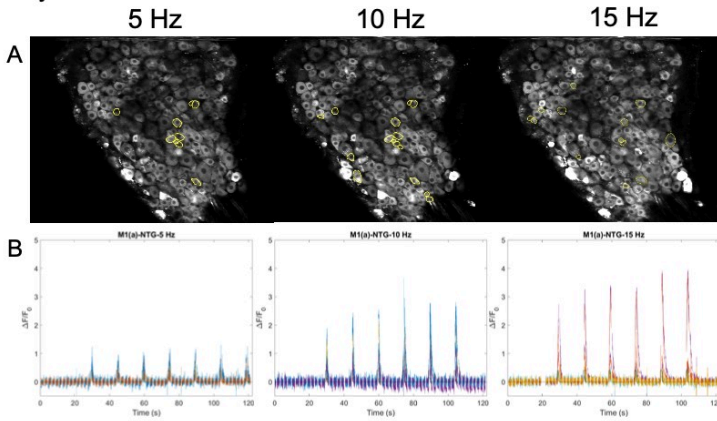
Figure 6: A. Images of DRG with circled ROI. B. Calcium fluorescence traces of responding neurons according to 5, 10, 15 Hz stimulation protocol.

Increased baseline sensitivity for tactile behavior in the chronic NTG model

NTG at 10 mg/kg, or vehicle, every other day for five times over a total period of nine days was injected intraperitoneally in the chronic model. Baseline measurements of mechanical sensitivity were carried out before NTG administration on days 1, 5, and 9 (Figure 7A). Days five and nine showed a significant reduction in paw withdrawal threshold, indicating the persistent hypersensitization induced by chronic NTG dosage (Two-way ANOVA: NTG days 5 and 9, p=0.04 and p=0.03 respectively). However, one- hour post-treatment responses showed sensitization in both the vehicle and NTG groups (p=0.06, Two-way ANOVA) (Figure 7B).
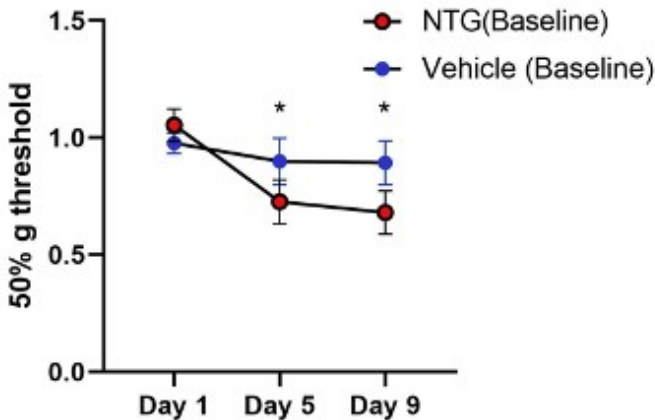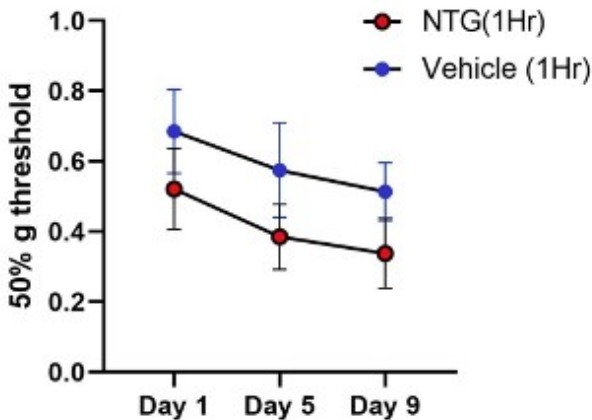
Figure 7: Tactile behavior in chronic NTG treatment model on Ai95/Pirt-1 mice. A. Baseline paw withdrawal thresholds before NTG treatment (10 mg/kg) on days 1, 5, and 9. (N=7 mice in NTG group, N=9 mice in vehicle control group). The baseline values on Day 5 and Day 9 were significantly different

than Day 1 in the NTG group and not in vehicle group (p=0.04 day 5, p=0.03 day 9, Two-way ANOVA). B. Paw withdrawal thresholds in chronic NTG and vehicle groups at 1-hr post-treatment. NTG withdrawal thresholds compared to vehicle group showed no significant difference (p=0.06, Two- way ANOVA).

Increased tactile sensitivity in acute SNP treated mice

Our behavior assays with acute SNP treatment showed a clear distinction in tactile sensitivity between the compound treated group and vehicle control (Figure 8). To optimize our acute SNP dosage for our imaging prep, we tested two doses of SNP: 2 mg/kg (p=0.02, Two-way ANOVA) (Figure 8A) and 2.5 mg/kg (p=0.13, Two-way ANOVA) (Figure 8C). Our goal was to identify the ideal dose that induces nociceptive effects without confounding the compound's vasodilatory effects. Additionally, SNP releases cyanide during metabolism, and cyanide toxicity is a potential, though rare, complication (Rindone & Sloane, 1992). Since both doses induced tactile sensitivity as recorded by the von Frey test, 2 mg/kg was chosen for our acute experiments involving in vivo imaging of C2 DRG neurons to reduce risk while still giving a behavioral response.
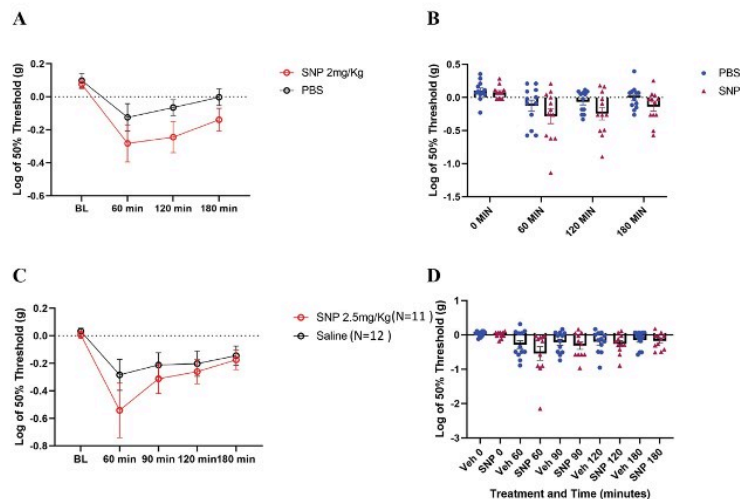
Figure 8: Tactile behavior assay outcomes in C57BL/6 mice treated with SNP. A/B. Paw withdrawal thresholds from N=12 mice each in SNP (2 mg/kg dose) and vehicle. (p=0.02, Two-way ANOVA). C/D. Paw withdrawal thresholds in SNP treated (2.5 mg/kg dose) and vehicle treated mice (SNP N=11 mice; vehicle N=12 mice) (p=0.13, Two-way ANOVA).

C2 DRG responses in the chronic and acute NTG models show subtle differences Chronic NTG model – In vivo GCaMP calcium fluorescence imaging for Ai95/Pirt1-Cre mice C2 DRG from chronic NTG and vehicle groups were completed within the first five days following the last intraperitoneal injection day (day 9). This ensured that the experiments were performed within the chronic NTG-induced sensitization period as reported by other groups (Moye & Pradhan, 2017). Calcium transient responses were recorded from DRG cells according to the stimulation paradigms.
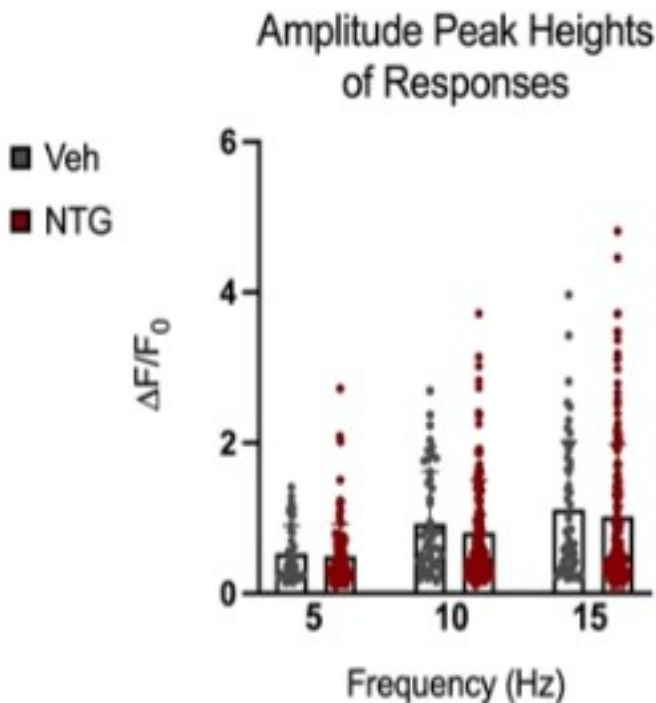
Figure 9: Comparison of peak amplitudes of calcium responses in C2 DRG of chronic NTG/Vehicle treated mice. Responses to six repeat stimulation trains at 5Hz, 10Hz, and 15Hz frequencies of 0.3mA current pulses. The ROIs from all the responding cell populations are plotted (NTG group, N=163 cells, n=14 mice; Vehicle group, N=54 cells, n=10 mice). $\Delta F/F0=(F-F0)/F0$

Peak amplitudes of calcium fluorescence in both NTG and vehicle groups were analyzed (Figure 9). No major differences were observed in response peak amplitudes between the chronic NTG group and the vehicle group. Ca2+ responses to C2 DRG stimulation in the chronic migraine model mice versus control vehicle administered mice were also evaluated

for expansion of responding cells. When in a nitroglycerine-induced chronic migraine state, a greater percentage of sensory neurons in the DRG respond when the greater occipital nerve dermatome is electrically stimulated ($p<0.05$, unequal variance t-test) (Figure 10).
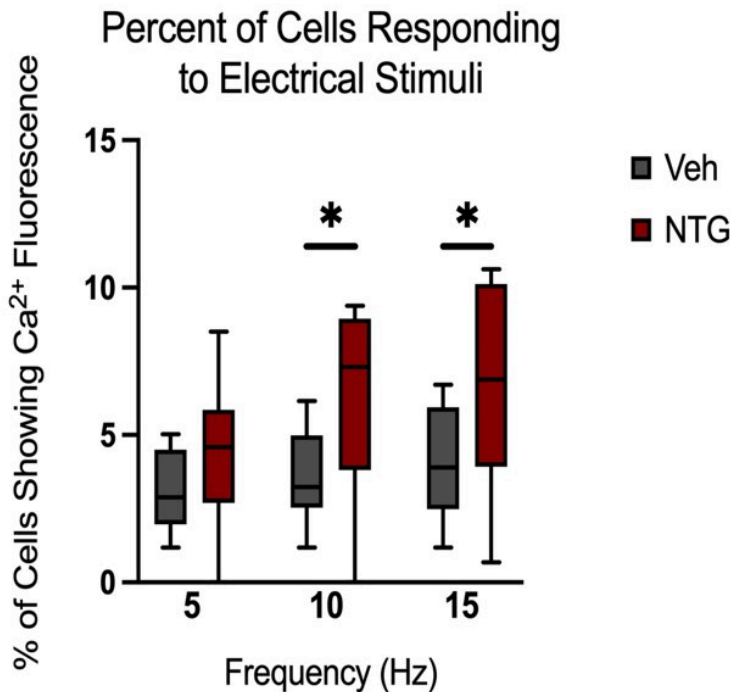
Figure 10: Comparison of the percentage of cells showing fluorescence (Vehicle n=10 mice, NTG n=14 mice). Responses to six repeat stimulation trains at 5Hz, 10Hz, and 15Hz frequencies of 0.3mA current pulses ($p<0.05$, unequal variance t-test).

Acute NTG model – A single dose of NTG (10 mg/kg) or vehicle was administered retro- orbitally during an in vivo imaging session. Responses to stimulation paradigms were collected before and after NTG or vehicle injection. The data

was analyzed as an average of all response traces between NTG and vehicle groups. We found subtle differences between the NTG pre-treatment response and the NTG post-treatment response (p=0.06, Two-way ANOVA) (Figure 11C). The vehicle group did not show any differences between pre-treatment amplitudes and post-treatment values (Figure 11D).
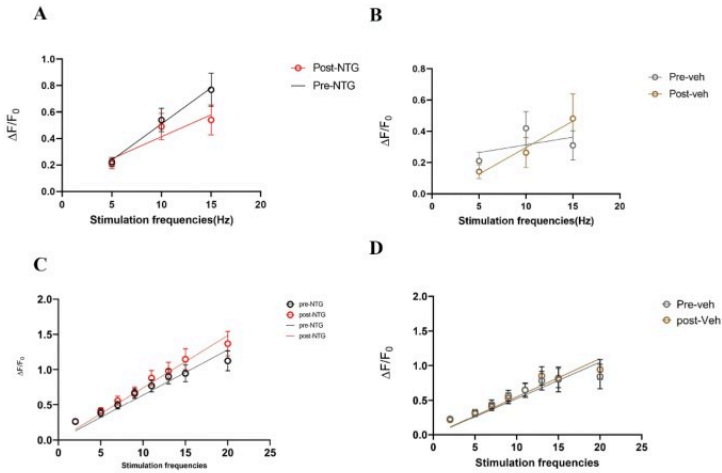
Figure 11: Comparison of peak amplitudes of calcium responses in C2 DRG of acute NTG/Vehicle treated mice to two stimulation paradigms. A. Responses to six repeat stimulation trains at 5Hz, 10Hz, and 15Hz frequencies of 0.3mA current pulses in the NTG treated mice. The mean±SEM of all ROIs from all the responding cell populations are plotted (NTG group, N=5 mice). B. Responses to six repeat stimulation trains in the vehicle group (Vehicle group, N=5 mice). C. Responses to range of frequencies from low 2Hz to high 20Hz with 0.3mA current pulses in the NTG group (p=0.06, Two-way ANOVA). D. Responses to 2-20Hz range of frequencies in the acute vehicle group.

Expansion of responders post-SNP treatment

Our behavior assays with acute SNP treatment showed a clear distinction in tactile sensitivity between the compound treated group and the vehicle control group (PBS/saline) (Figure 8). A dose of 2 mg/kg SNP was delivered retro-orbitally while concurrently imaging the C2 DRG. Responses to stimulation paradigms were collected before and after SNP or vehicle injection, and the data was analyzed for response peak amplitudes and the expansion of responding cells. We did not see a significant change in the response amplitude of responding cells to the frequency ranges of 5–15 Hz. Interestingly, our preliminary observations show an increase in the number of new responding cells in the SNP treated group (Figures 12–14). We compared the response traces from the same cell ROIs within an FOV before retroorbital administration of SNP and after. The new responders had low amplitude responses and were mostly identifiable at higher frequencies of 10 and 15 Hz (N=6 mice for SNP group, N=4 for vehicle group).
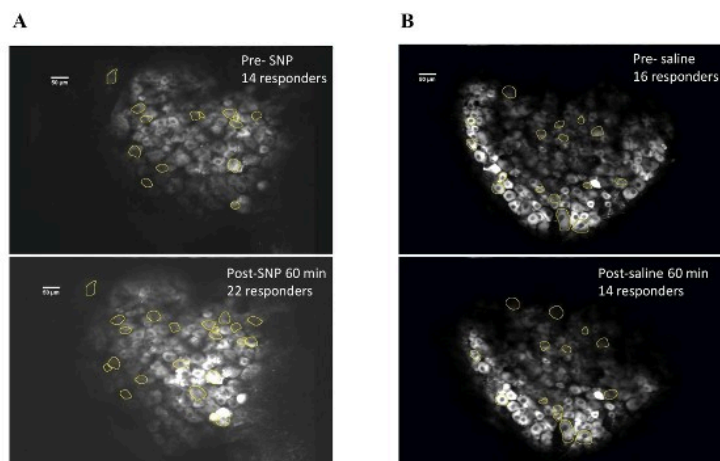
Figure 12: DRG cell responders before and after SNP/Vehicle

treatment. A. Upper panel shows a representative FOV of responders before SNP and lower panel shows same FOV with responders post retroorbital administration of SNP. B. Upper panel shows an FOV before vehicle and lower panel shows same FOV after vehicle PBS administration in the retroorbital space.
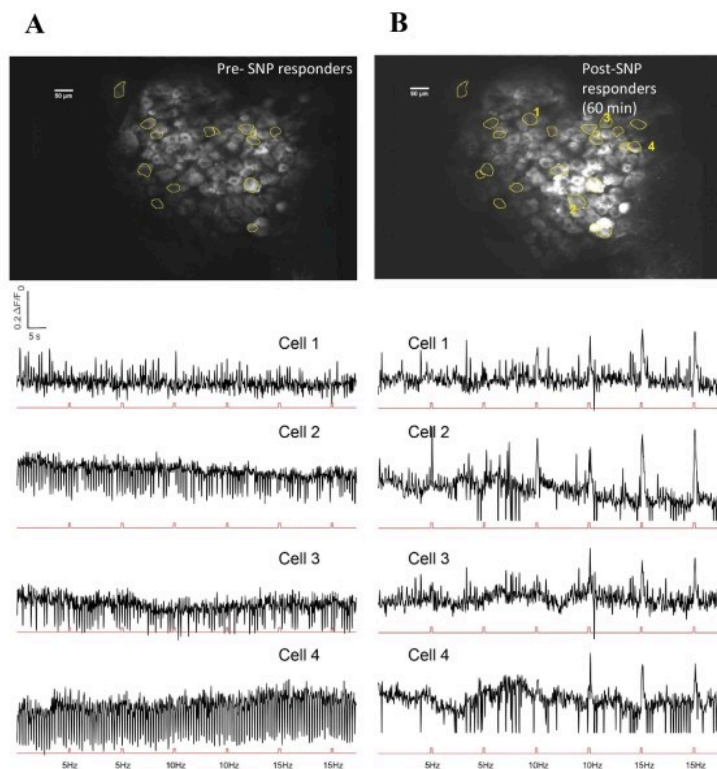
Figure 13: Representative image of DRG neurons and response traces to electrical stimuli before and after SNP. A. Upper panel shows FOV with responder cells before SNP. Lower panel has selected cell traces corresponding to those marked in B (Cells 1-4 in B). Stimulation pulses are represented in the red trace below each cell trace. B. Upper panel has

same FOV after SNP administration, with representative new responders marked. Lower panel has corresponding responder peaks aligned to stimulation pulse sets shown below each cell trace.
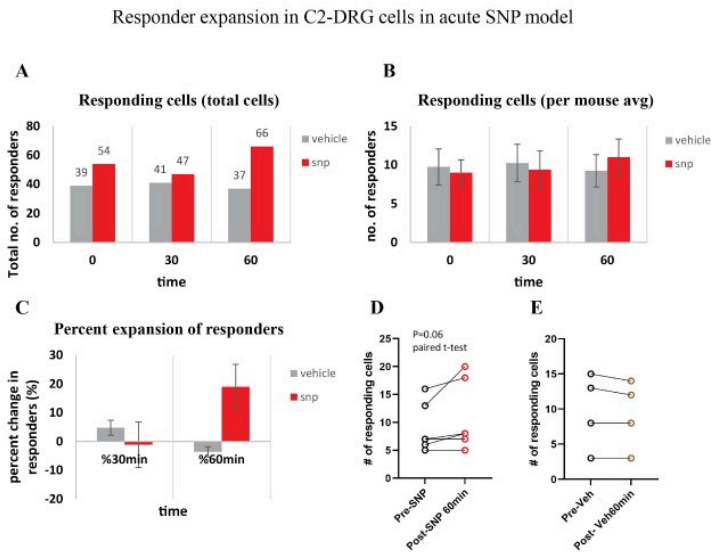
Figure 14: Responder expansion in cervical DRG in SNP treated mice. A. Total responding cells before SNP/Vehicle treatment (0 min), at 30-min and 60-min post-treatment. N=6 mice for SNP group, N=4 for vehicle group. B. Average of responding cells per mouse in each SNP or vehicle group. C. Percentage of responder increase at 30 min and 60 min compared to before treatment. D. Comparison of the number of responder cells from each mouse plotted before and after treatment with SNP (p=0.06, paired t-test). E. Comparison of the number of responder cells from each mouse plotted before and after treatment with vehicle.

DISCUSSION

Migraine is a disease that primarily affects the sensory

nervous system. Along with other symptoms, migraine is characterized by attacks of unilateral, throbbing craniofacial pain. This pain is thought to be the consequence of activation of the trigeminovascular system and the dorsal root ganglia of cervical roots C1–C3 (Brennan & Pietrobon, 2018). We set out to investigate the ascending circuit pathway that may be involved in the manifestation of the migraine pain phenotype. We started our study of the nociceptive circuit at the C2 DRG due to surgical accessibility and compared neuronal activity changes in both chronic and acute headache models.

NTG is an effective migraine trigger in humans (Karsan et al., 2017). It has also been established as a model of chronic migraine-associated pain in mice (Moye & Pradhan, 2017). Indeed, we observe progressive and sustained mechanical allodynia at baseline following a chronic NTG treatment model, indicating persistent hypersensitization. Similar tactile allodynia results are seen with an acute SNP-induced headache model (Avona et al., 2020; Wattiez et al., 2021). This behavioral assay is consistent with the cutaneous allodynia reported in many migraine patients (Burstein et al., 2015).

Our experience with NTG encountered confounding variables. We see persistent tactile sensitization behavior in mice that underwent chronic dosing of NTG; however, one-hour posttreatment responses show sensitization in both vehicle and NTG groups. We think that the vehicle composition for NTG is a potential confound. The vehicle composition for NTG contains 30% propylene glycol and 30% alcohol. Propylene glycol has been shown to activate transient receptor potential (TRP) channels (Niedermirtl et al., 2018). Activation of TRP channels promotes excitation of nociceptive afferent fibers that potentially lead to pain (Dussor et al., 2014). Alcohol can activate TRPV1 channels that provide a sensation of heat and

pain (Trevisani et al., 2002). Hence, the nociceptive effects induced by propylene glycol and alcohol could be a potential reason for the noisy behavior in both NTG and vehicle treated mice after one hour. The time course of our acute experiments also falls within this time span of activation and excitation, which may have negatively affected the outcomes.

We observe behavioral hypersensitivity to pain in the chronic NTG model. When we examine how C2 DRG neurons respond to the NTG challenge (hypersensitivity), we observe increases in the number of neurons responding to stimulation, indicating a change in the sensitivity of neurons at the C2 level. While observations in the chronic model show an expansion of responders in correlation with administration of NTG, this leads to the need for an acute headache model. The acute model allows us to investigate the effect of drug administration after establishing a baseline on the same animal, minimizing confounding variables. Similar patterns are seen in the preliminary data of acute models with the expansion of responders post administration of SNP.

Even though we did not see significant changes in the response amplitude of responding cells after acute administration of SNP, our initial observations show an increase in the number of new responding cells in the SNP treated group. This increased sensory neuron activation could be correlated with pain behaviors (Zheng et al., 2022), as more sensory neuron responses could mean potential amplification of signals in the ascending circuitry, meaning increased sensitivity. This phenotype appears to be similar to what has been seen in other pain models. Studies found that peripheral neuropathic pain models and complete Freund Adjuvant (CFA)-induced inflammatory pain models show an increase in activated neurons in L4 DRG surgical preparations post nerve

injury or administration of CFA (Kim et al., 2016). These studies involved longer, chronic exposure to nerve injury or inflammation than our acute model, which could account for the lower extent of the responder expansion in our observations. While these results have been shown in other pain models, to our best knowledge, we offer the first results in a cervical DRG.

The low magnitude of calcium fluorescence changes we observe in the chronic NTG or acute NTG and SNP treatment models may align with the view that sensory neurons act as faithful relay centers, resisting major changes in activation. The all-or- nothing law is an important principle: no matter how weak the noxious stimuli are, once it reaches a certain threshold, the sensory neuron action potential will fire at full strength (Adrian, 1914). However, the lack of major increase in calcium fluorescence trace amplitude does not imply an absence of sensory changes. Furthermore, we do see subtle changes in the number of responding cells, which could have amplified effects at the dorsal horn or brainstem level that we have proposed to investigate. In vivo DRG imaging is the starting point for studying the role of primary sensory neurons in different somatosensations, including pain. It may thus be even more important to identify the origin of these changes in DRG because of targeting, which could lead to more specific therapies that prevent downstream amplification of sensory processing.

We ultimately aim to dissect the role of specific cell types within the C2 DRG involved in headache phenotypes of varying origins. Our experiments are currently limited in assessing the sensory neuronal classes that may be involved in the migraine pain mechanisms. For example, our electrical stimulation paradigm targeting the greater occipital nerve

(GON) trunk region could activate multiple neuronal types relaying a mix of sensory modalities—not just pain. Additional experiments involving defined sensory modalities to probe specific cell types involved and their respective receptive fields would allow us to delineate peripheral circuitry critically involved in the migraine pain phenotype.

This project lays the groundwork for many subsequent questions. Future directions include performing C2 DRG afferent cell subtype-specific studies, identifying migraine relevant alterations in C2 DRG in acute models of migraine, studying migraine relevant alterations in DRG to CNS synapses, and investigating sex differences in migraine responses at a cellular level. Importantly, the understanding of nociceptive network activation in migraine will allow us to look at changes in the pain circuit that serve as markers for a migraine attack, observe how they occur, and see if they can be reversed at a neuron or circuit level to prevent the pain associated with migraine.

REFERENCES

Adrian, E. D. (1914). The all-or-none principle in nerve. J Physiol, 47(6), 460-474. https://doi.org/10.1113/jphysiol.1914.sp001637

Anderson, M., Zheng, Q., & Dong, X. (2018). Investigation of Pain Mechanisms by Calcium Imaging Approaches. Neurosci Bull, 34(1), 194-199. https://doi.org/10.1007/s12264-017-0139-9

Arcilla, C. K., & Tadi, P. (2022). Neuroanatomy, Unmyelinated Nerve Fibers. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK554461/

Ashina, S., Foster, S. A., Nicholson, R. A., Araujo, A. B., Reed, M. L., Shapiro, R. E., Buse, D. C., Jaffe, D. H., Cambron-Mellott, M., Li, V. W., Zagar, A., Pearlman, E. M., & Lipton, R. B. (2019). Opioid Use Among People with Migraine: Results of

the OVERCOME Study. Headache, 59(S1), 208. https://doi.org/ https://doi.org/10.1111/head.13549

Avona, A., Mason, B. N., Lackovic, J., Wajahat, N., Motina, M., Quigley, L., Burgos- Vega, C., Moldovan Loomis, C., Garcia-Martinez, L. F., Akopian, A. N., Price, T. J., & Dussor, G. (2020). Repetitive stress in mice causes migraine-like behaviors and calcitonin gene-related peptidedependent hyperalgesic priming to a migraine trigger. Pain, 161(11), 2539-2550. https://doi.org/ 10.1097/j.pain.0000000000001953

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2016). Neuroscience: Exploring the Brain (Fourth Edition ed.). Wolters Kluwer. Bektas, F., & Soyuncu, S. (2010). Nitroglycerin-induced migraine type headache: bilaterally visible temporal arteries. Int J Emerg Med, 3(4), 463-464. https://doi.org/10.1007/ s12245-010- 0248-y

Brennan, K. C., & Pietrobon, D. (2018). A Systems Neuroscience Approach to Migraine. Neuron, 97(5), 1004-1021. https://doi.org/10.1016/j.neuron.2018.01.029

Burstein, R., Noseda, R., & Borsook, D. (2015). Migraine: multiple processes, complex pathophysiology. J Neurosci, 35(17), 6619-6629. https://doi.org/10.1523/JNEUROSCI.0373-15.2015

Byrne, J. H. (1997). Introduction to Neurons and Neuronal Networks Castro-Junior, C., Ferreira, L., Delgado, M., Silva, J., & Santos, D. (2018). Role of Calcium Permeable Channels in Pain Processing. Intechopen. https://doi.org/10.5772/ intechopen.77996

Caylor, J., Reddy, R., Yin, S., Cui, C., Huang, M., Huang, C., Ramesh, R., Baker, D. G., Simmons, A., Souza, D., Narouze, S., Vallejo, R., & Lerman, I. (2019). Spinal cord stimulation in chronic pain: evidence and theory for mechanisms of action. Bioelectron Med, 5. https://doi.org/10.1186/s42234-019-0023-1

Chen, C., Zhang, J., Sun, L., Zhang, Y., Gan, W. B., Tang, P., & Yang, G. (2019). Long- term imaging of dorsal root ganglia in awake behaving mice. Nat Commun, 10(1), 3087. https://doi.org/10.1038/s41467-019-11158-0

Chen, L., Guo, T., Zhang, S., Smith, P. P., & Feng, B. (2022). Blocking peripheral drive from colorectal afferents by subkilohertz dorsal root ganglion stimulation. Pain, 163(4), 665-681. https://doi.org/10.1097/j.pain.0000000000002395

Chowdhury, D., Datta, D., & Mundra, A. (2021). Role of Greater Occipital Nerve Block in Headache Disorders: A Narrative Review. Neurol India, 69(Supplement), S228-S256. https://doi.org/10.4103/0028-3886.315993 C

oppola, G., & Schoenen, J. (2012). Cortical excitability in chronic migraine. Curr Pain Headache Rep, 16(1), 93-100. https://doi.org/10.1007/s11916-011-0231-1

Cowan, W. M., Harter, D. H., & Kandel, E. R. (2000). The emergence of modern neuroscience: some implications for neurology and psychiatry. Annu Rev Neurosci, 23, 343-391. https://doi.org/10.1146/annurev.neuro.23.1.343

de Prado, B. M., & Russo, A. F. (2006). CGRP receptor antagonists: A new frontier of antimigraine medications. Drug Discov Today Ther Strateg, 3(4), 593-597. https://doi.org/10.1016/j.ddstr.2006.11.003

Deuis, J. R., Dvorakova, L. S., & Vetter, I. (2017). Methods Used to Evaluate Pain Behaviors in Rodents. Front Mol Neurosci, 10, 284. https://doi.org/10.3389/fnmol.2017.00284

Divakaran, S., & Loscalzo, J. (2017). The Role of Nitroglycerin and Other Nitrogen Oxides in Cardiovascular Therapeutics. Journal of the American College of Cardiology, 70(19), 2393-2410. https://doi.org/https://doi.org/10.1016/j.jacc.2017.09.1064

Dodson, H., Bhula, J., Eriksson, S., & Nguyen, K. (2018). Migraine   Treatment   in   the   Emergency   Department:

Alternatives to Opioids and their Effectiveness in Relieving Migraines and Reducing Treatment Times. Cureus, 10(4), e2439. https://doi.org/10.7759/cureus.2439

Dussor, G., Yan, J., Xie, J. Y., Ossipov, M. H., Dodick, D. W., & Porreca, F. (2014). Targeting TRP channels for novel migraine therapeutics. ACS Chem Neurosci, 5(11), 1085-1096. https://doi.org/10.1021/cn500083e

Edvinsson, J. C. A., Vigano, A., Alekseeva, A., Alieva, E., Arruda, R., De Luca, C., D'Ettore, N., Frattale, I., Kurnukhina, M., Macerola, N., Malenkova, E., Maiorova, M., Novikova, A., Rehulka, P., Rapaccini, V., Roshchina, O., Vanderschueren, G., Zvaune, L., Andreou, A. P., . . . European Headache Federation School of Advanced, S. (2020). The fifth cranial nerve in headaches. J Headache Pain, 21(1), 65. https://doi.org/10.1186/s10194-020-01134-1

Feldman, D., & Scott, K. (2020). Editorial overview: Systems neuroscience. Curr Opin Neurobiol, 64, iii. https://doi.org/10.1016/j.conb.2020.10.011 Goadsby, P. J., Holland, P. R., Martins-Oliveira, M., Hoffmann, J., Schankin, C., & Akerman, S. (2017). Pathophysiology of Migraine: A Disorder of Sensory Processing. Physiol Rev, 97(2), 553-622. https://doi.org/10.1152/physrev.00034.2015

Handler, A., & Ginty, D. D. (2021). The mechanosensory neurons of touch and their mechanisms of activation. Nat Rev Neurosci, 22(9), 521-537. https://doi.org/10.1038/s41583-021-00489-x

Headache Classification Committee of the International Headache Society, I. (2013). The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia, 33(9), 629-808. https://doi.org/10.1177/0333102413485658

Hodge, R. D., Bakken, T. E., Miller, J. A., Smith, K. A., Barkan, E. R., Graybuck, L. T., Close, J. L., Long, B., Johansen, N., Penn,

O., Yao, Z., Eggermont, J., Hollt, T., Levi, B. P., Shehata, S. I., Aevermann, B., Beller, A., Bertagnolli, D., Brouner, K., . . . Lein, E. S. (2019). Conserved cell types with divergent features in human versus mouse cortex. Nature, 573(7772), 61-68. https://doi.org/10.1038/s41586- 019-1506-7

Jensen, R., & Stovner, L. J. (2008). Epidemiology and comorbidity of headache. Lancet Neurol, 7(4), 354-361. https://doi.org/10.1016/S1474-4422(08)70062-0

Jones, B. (2021). Why do we need to use animals in neuroscience research? Retrieved April 26 from https://www.eara.eu/post/feature-why-do-we-need-to-use-animals-   in-neuroscienceresearch#:~: text=While%20non%2Danimal%20methods%20of,study%20the %20 brain%20in%20action.

Karsan, N., Bose, P., Thompson, C., & Goadsby, P. (2017). PO067 Nitroglycerin triggering as a human migraine model in clinical research. Journal of Neurology, Neurosurgery, and Psychiatry. https://doi.org/10.1136/jnnp-2017-ABN.99

Kendroud, S., Fitzgerald, L. A., Murray, I., & Hanna, A. (2021). Physiology,   Nociceptive   Pathways.   StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK470255/

Kim, A. Y., Tang, Z., Liu, Q., Patel, K. N., Maag, D., Geng, Y., & Dong, X. (2008). Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell, 133(3), 475-485. https://doi.org/10.1016/j.cell.2008.02.053

Kim, Y. S., Anderson, M., Park, K., Zheng, Q., Agarwal, A., Gong, C., Saijilafu, Young, L., He, S., LaVinka, P. C., Zhou, F., Bergles, D., Hanani, M., Guan, Y., Spray, D. C., & Dong, X. (2016). Coupled Activation of Primary Sensory Neurons Contributes to Chronic Pain. Neuron, 91(5), 1085-1096. https://doi.org/10.1016/j.neuron.2016.07.044

Lipton, R. B., Bigal, M. E., Ashina, S., Burstein, R., Silberstein,

S., Reed, M. L., Serrano, D., Stewart, W. F., & American Migraine Prevalence Prevention Advisory, G. (2008). Cutaneous allodynia in the migraine population. Ann Neurol, 63(2), 148-158. https://doi.org/10.1002/ana.21211

May, A., & Schulte, L. H. (2016). Chronic migraine: risk factors, mechanisms and treatment. Nat Rev Neurol, 12(8), 455-464. https://doi.org/10.1038/nrneurol.2016.93

Moye, L. S., & Pradhan, A. A. A. (2017). Animal Model of Chronic Migraine-Associated Pain. Curr Protoc Neurosci, 80, 9 60 61-69 60 69. https://doi.org/10.1002/cpns.33

Neeb, L., Meents, J., & Reuter, U. (2010). 5-HT(1F) Receptor agonists: a new treatment option for migraine attacks? Neurotherapeutics, 7(2), 176-182. https://doi.org/10.1016/j.nurt.2010.03.003

Niedermirtl, F., Eberhardt, M., Namer, B., Leffler, A., Nau, C., Reeh, P. W., & Kistner, K. (2018). Etomidate and propylene glycol activate nociceptive TRP ion channels. Mol Pain, 14, 1744806918811699. https://doi.org/10.1177/1744806918811699

Nielsen, M. (2020). Advanced Human Anatomy (Seventh ed.). Kendall Hunt. Olesen, J., Burstein, R., Ashina, M., & Tfelt-Hansen, P. (2009). Origin of pain in migraine: evidence for peripheral sensitisation. Lancet Neurol, 8(7), 679-690. https://doi.org/10.1016/S1474-4422(09)70090-0

Opioids and Migraine. (2018). Retrieved March 28 from https://americanheadachesocie¬ty.org/news/opioids-migraine/ Park, J., & Luo, Z. D. (2010). Calcium channel functions in pain processing. Channels (Austin), 4(6), 510-517. https://doi.org/10.4161/chan.4.6.12869

Rindone, J. P., & Sloane, E. P. (1992). Cyanide toxicity from sodium nitroprusside: risks and management. Ann Pharmacother, 26(4), 515-519. https://doi.org/10.1177/106002809202600413

Society, I. H. (2013). The International Classification of Headache Disorders (ICHD-3). Cehpalalgia. https://doi.org/10.1177/0333102413485658

Sureda-Gibert, P., Romero-Reyes, M., & Akerman, S. (2022). Nitroglycerin as a model of migraine: Clinical and preclinical review. Neurobiology of Pain, 12. https://doi.org/https://doi.org/10.1016/j.ynpai.2022.100105

Todd, A. J. (2013). Nociceptive Circuitry in the Spinal Cord. Encyclopedia of Pain. https://doi.org/https://doi.org/10.1007/978-3-642-28753-4_2748

Tortora, G., & Nielsen, M. (2017). Principles of Human Anatomy, John Wiley and Sons.

Trevisani, M., Smart, D., Gunthorpe, M. J., Tognetto, M., Barbieri, M., Campi, B., Amadesi, S., Gray, J., Jerman, J. C., Brough, S. J., Owen, D., Smith, G. D., Randall, A. D., Harrison, S., Bianchi, A., Davis, J. B., & Geppetti, P. (2002). Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci, 5(6), 546-551. https://doi.org/10.1038/nn0602-852

Wattiez, A. S., Gaul, O. J., Kuburas, A., Zorrilla, E., Waite, J. S., Mason, B. N., Castonguay, W. C., Wang, M., Robertson, B. R., & Russo, A. F. (2021). CGRP induces migraine-like symptoms in mice during both the active and inactive phases. J Headache Pain, 22(1), 62. https://doi.org/10.1186/s10194-021-01277-9

Yam, M. F., Loh, Y. C., Tan, C. S., Khadijah Adam, S., Abdul Manan, N., & Basir, R. (2018). General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. Int J Mol Sci, 19(8). https://doi.org/10.3390/ijms19082164

Zheng, Q., Xie, W., Luckemeyer, D. D., Lay, M., Wang, X. W., Dong, X., Limjunyawong, N., Ye, Y., Zhou, F. Q., Strong, J. A., Zhang, J. M., & Dong, X. (2022). Synchronized cluster firing, a

distinct form of sensory neuron activation, drives spontaneous pain. Neuron, 110(2), 209-220 e206. https://doi.org/10.1016/j.neuron.2021.10.019

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57. Research Reflection by Kara Andersen

Kara Andersen

Faculty Mentor: K.C. Brennan (Neurology, University of Utah)

My undergraduate research experience was incredible. My mentor took a chance on the undergraduate and fostered in me an insatiable desire to understand the inner-workings of the brain. I was encouraged to always think more ambitiously and outside of the box to accomplish my goals and more. I was provided with opportunities to learn about new topics and gain a deeper understanding of existing concepts. I was required to analyze and evaluate information, identify patterns and relationships, and draw conclusions based on evidence. Most importantly, this experience instilled confidence in me during this challenging but rewarding time. I am excited to use all that I have learned on my future continuation with education in medical school.

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58. **Control of Isolation Induced Aggression through Activation of Medial Prefrontal Cortex Pyramidal Neurons**

Jordyn Gagon; Rachel E. Gatlin (Neurobiology and Anatomy); and Moriel Zelikowksy (Neurobiology and Anatomy)

Faculty Mentor: Moriel Zelikowksy (School of Medicine, University of Utah)

Background

The effects of social isolation have recently been felt deeply across the globe as the world seemingly shut down in response to the novel Covid-19 pandemic. While social isolation is critical in slowing disease spread, social interaction is critical for human health. Social isolation has been linked to poor health, depression, and overall decline in well-being (Courtin and Knapp, 2015). The effects of social isolation have been seen before, as prison inmates who have been placed in solitary confinement are more likely to experience aggression, suicidal ideology, and irritability (Scharf Smith, 2006). Furthermore, isolated mice show increased aggression compared to group housed mice (Zelikowsky et. al., 2018).

The prefrontal cortex (PFC), which is involved in emotional and cognitive regulation, is a target area of the brain in controlling aggression. Damage to the PFC can result is severe emotional disruption, often leading to aggression. Patients with penetrating traumatic brain injury with lesions specific to PFC territories displayed the highest levels of aggression compared to patients with lesions in other parts of the brain. In a study examining patients with penetrating traumatic brain injury, patients with the highest levels of aggression primarily presented lesions in the PFC region (Pardini et. al., 2011). One historic example of this damage is Phineas Gage, a railroad worker who experienced an injury in which a rod penetrated his skull and into the PFC. Following the injury, Phineas Gage became angry and showed poor social judgement (Siever, 2008).

Previous work has established that medial prefrontal cortex (mPFC) pyramidal neurons project to multiple subcortical aggression centers (Biro et. al., 2018), and that inhibition of such neurons in mPFC reduces aggression (Takahashi et. al.

2014; Van Heukelum et. al, 2021). This work has led to a model of the mPFC controlling aggression through these pyramidal neurons in which activation of mPFC pyramidal neurons in aggressive mice reduces aggression and inhibition of mPFC pyramidal neurons seems to enhance aggression. Despite this, no work has focused on how this population of neurons may regulate social isolation-induced aggression nor have these neurons been studied in females. Here, we expand on previous work showing a link between decreased mPFC pyramidal neuron activity and aggression in males by examining whether activation of mPFC pyramidal neurons reduces aggression in socially isolated female mice. We hypothesize that activation of mPFC pyramidal neurons in socially isolated female animals will reduce aggressive behavior.

Methods

A Designer Receptor Exclusively Activated by Designer Drugs (DREADD)-mediated approach was taken to activate the mPFC pyramidal neurons. To this end we infused a virus encoding the excitatory DREADD, hM3D, fused to mCherry, under control of the CaMKII promoter into the mPFC of female C57Bl6/N mice (N=8). This will express the activating DREADD in the pyramidal neurons of the mPFC. A virus expressing mCherry without the hM3D DREADD under the control of the CaMKII promoter was used as a control (N=9). Following surgery, all mice were isolated (single housed) for four weeks to induce aggression. Each animal was then tested on the resident intruder assay in which a novel age and sex matched docile mouse is introduced into the home cage of the test animal. Each test animal was tested twice, once in which the DREADD ligand, Descholorclozapine (DCZ) administered via i.p, injection and once with vehicle solution, 48 hours apart. The viral conditions were counterbalanced such that half the

animals in each viral condition (hM3D or mCherry control) received DCZ on the first test day and half received an injection of the vehicle on the first test day. High-speed video was recorded during the resident intruder assay, which was then used to score aggressive (attack) and social behaviors (face investigation, anogenital investigation, and body investigation) using Noldus Observer. Primarily, attack behavior was defined as the test animal tussling, biting, and kicking the intruder. Mounting is defined when the test animal is on top of the intruder, but not kicking or biting. Lastly, anogenital investigation is defined as the test animal sniffing the rear end of the intruder. Upon the conclusion of testing, all brains were extracted, sectioned, and imaged to confirm virus expression in the mPFC (Figure 1).

Figure 1 shows virus (AAV2-CaMKII-hM3D-mCherry) confirmation in the mPFC brain region.
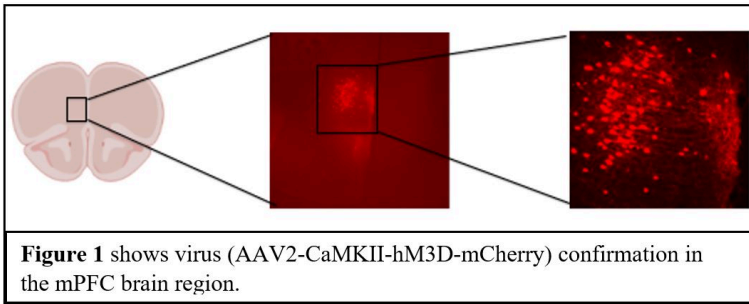
Results

DREADD-mediated activation of mPFC pyramidal neurons significantly decreased attack aggression (Repeated Measures ANOVA, $p<0.05$) (Figure 2). However, activation of mPFC pyramidal neurons had no effect on other social behaviors, including mounting ($p=0.2079$) (Figure 3) nor anogenital investigation ($p=.9955$) (Figure 4). Furthermore, there were no significant results within the control mCherry condition, suggesting that DCZ did not have any effect on behavior in the absence of the DREADD.
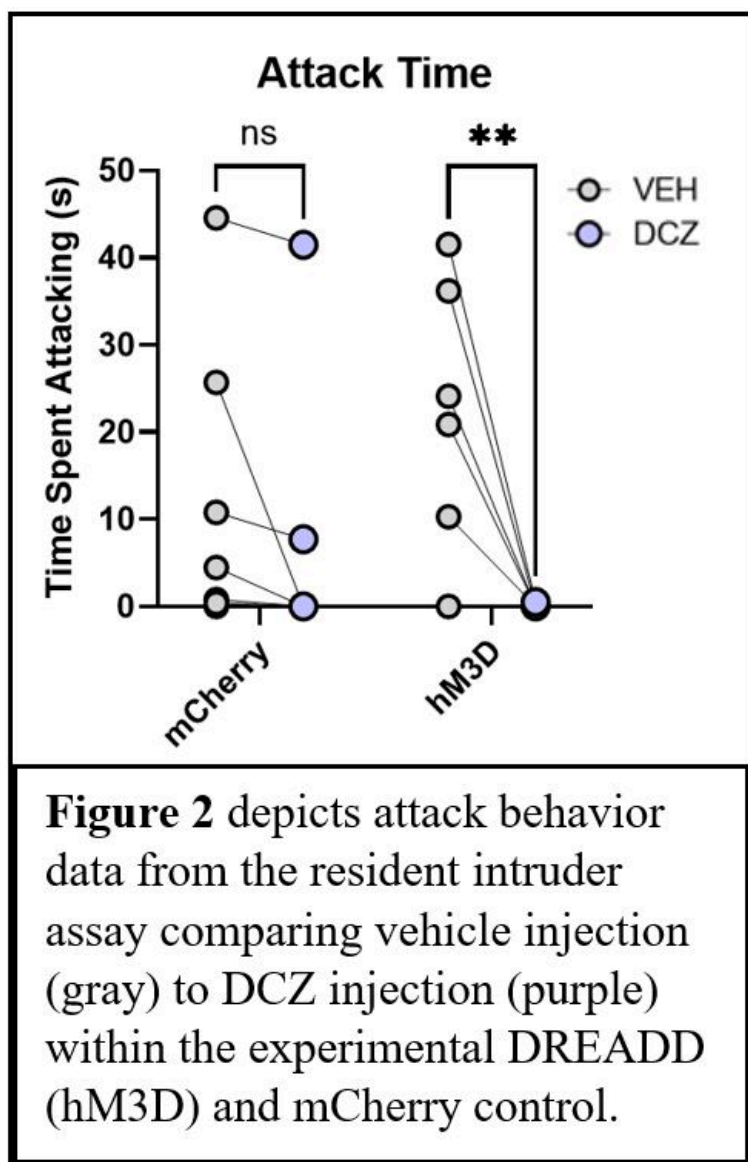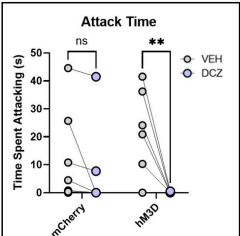
Figure 2 depicts attack behavior data from the resident intruder assay comparing vehicle injection (gray) to DCZ injection (purple) within the experimental DREADD (hM3D) and mCherry control.

Figure 2 depicts attack behavior data from the resident intruder assay comparing vehicle injection (gray) to DCZ injection (purple) within the experimental DREADD (hM3D) and mCherry control.
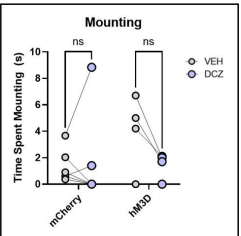
Figure 3 depicts mounting behavior data from the resident intruder assay comparing vehicle injection (gray) to DCZ injection (purple) within the experimental DREADD (hM3D) and mCherry
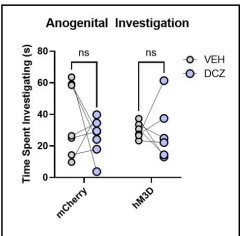
Figure 4 depicts anogenital investigation data from the resident intruder assay comparing vehicle injection (gray) to DCZ injection (purple) within the experimental DREADD (hM3D)

Conclusions

These results support the hypothesis that the mPFC plays an inhibitory role in aggressive behavior. Additionally, mPFC pyramidal neurons are essential to this inhibitory control. Activation of mPFC pyramidal neurons seems to exclusively alter aggression and does not affect other social behaviors, such as anogenital investigation. Next steps include activating mPFC pyramidal neurons in male mice to compare differences across sex.

Acknowledgements

References

Biro, Laszlo, et al. "Task Division within the Prefrontal Cortex: Distinct Neuron Populations Selectively Control

Different Aspects of Aggressive Behavior via the Hypothalamus." *The Journal of Neuroscience*, vol. 38, no. 17, 2018, pp. 4065–4075., https://doi.org/10.1523/jneurosci.3234-17.2018.

Courtin, Emilie, and Martin Knapp. "Social Isolation, Loneliness and Health in Old Age: A Scoping Review." *Health & Social Care in the Community*, vol. 25, no. 3, 2015, pp. 799–812., https://doi.org/10.1111/hsc.12311.

Pardini, M., et al. "Prefrontal Cortex Lesions and MAO-A Modulate Aggression in Penetrating Traumatic Brain Injury." *Neurology*, vol. 76, no. 12, 2011, pp. 1038–1045., https://doi.org/10.1212/wnl.0b013e318211c33e.

Siever, Larry J. "Neurobiology of Aggression and Violence." *American Journal of Psychiatry*, vol. 165, no. 4, 2008, pp. 429–442., https://doi.org/10.1176/appi.ajp.2008.07111774.

Smith, Peter Scharff. "The Effects of Solitary Confinement on Prison Inmates: A Brief History and Review of the Literature." *Crime and Justice*, vol. 34, no. 1, 2006, pp. 441–528., https://doi.org/10.1086/500626.

Takahashi, Aki, et al. "Control of Intermale Aggression by Medial Prefrontal Cortex Activation in the Mouse." *PLoS ONE*, vol. 9, no. 4, 2014, https://doi.org/10.1371/journal.pone.0094657.

Van Heukelum, Sabrina, et al. "A Central Role for Anterior Cingulate Cortex in the Control of Pathological Aggression." *Current Biology*, vol. 31, no. 11, 2021, https://doi.org/10.1016/j.cub.2021.03.062.

Zelikowsky, Moriel, et al. "The Neuropeptide TAC2 Controls a Distributed Brain State Induced by Chronic Social Isolation Stress." *Cell*, vol. 173, no. 5, 2018, https://doi.org/10.1016/j.cell.2018.03.037.

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59. Correlation of White Matter Hyperintensities with Physical Activity and Diet

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Faculty Mentor: Adam de Havenon (Neurology, University of Utah)

Objective: Our aim is to identify a correlation between level of physical activity and nutritional intake that indicates significant white matter hyperintensity (WMH) development in the brain.

Background: White matter hyperintensities are a radiographic marker of cerebral small vessel disease (CSVD). Individual's levels of physical activity and nutritional intake have been hypothesized to be involved in the pathogenesis of WMH, although prior results have been inconsistent. Regular exercise can significantly reduce the risk of developing dementia and a diet rich in fiber and antioxidants can have similar effects. We will be performing a cross sectional analysis of the association between nutritional data obtained from an Automated Self-Administered 24-Hour Dietary Assessment Tool and physical activity data obtained from the International Physical Activity Questionnaire and WMH on a brain MRI.

Method: A retrospective chart review conducted on adults with a brain MRI from the EPIC database. The primary outcome was the burden of WHM measured on the Fazekas score, as a total score and either periventricular (PVWM) or deep white matter (DWM). The Fazekas score will be read with the individual predictors of the brain MRI measurements and adjusted for potential confounders such as patient age, sex, and hypertension.

Results: We identified patients aged 60 years or older who had a brain MRI done within the last year and were capable of all activities of daily living. We will categorize patients based on their level of physical activity and quality of nutritional intake against their WMH Fazekas score. Conclusion Physical activity and nutritional intake might help determine the extent of WMH in an individual. Discovering a relationship in asymptomatic patients could give vital information for

understanding the cause of WMH which can have dramatic effects on the aging population.

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60. **Research Reflection by Gauri Garg**

Gauri Garg

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My research experience has been an instrumental aspect of my professional and educational development as an undergraduate student. I have done research through the Department of Neurology, Department of Gastroenterology, through the Pediatric Clinical Research Minor, and at a summer internship at the National Institutes of Health. These experiences have taught me the scientific process, interacting with research subjects, submitting an IRB application, presenting at conferences, and writing scientifically. In the future, I hope to become a physician and pursue research in my profession. Opportunities through doing undergraduate research have given me leadership positions in addition to research experience. I have worked as an Undergraduate

Research Leader through the Office of Undergraduate
Research and helped mentor other students trying to get
involved in research. These opportunities will help me achieve
my goals and be a leader as a physician.

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61. Challenges Locating the Scene of Emergency: A Qualitative Study of the EMS System in Rwanda

McKenna Hunt; Sudha Jayaraman (Surgery); Melissa H. Watt (Population Health Sciences); Mediatrice Niyonsaba; Jean Marie Uwitonze; Justine Davies; Rebecca Maine; and Menelas Nkeshimana

Faculty Mentor: Sudha Jayaraman (Surgery, University of Utah)

Introduction: Efficient pre-hospital emergency care can significantly improve healthcare outcomes. One significant challenge that contributes to inefficiency in pre-hospital care is difficultly locating the patient requiring emergency care. The goal of this study was to describe challenges emergency medical services (EMS) teams face in Rwanda locating emergencies, and to explore potential opportunities for improvement.

Methods: Between August 2021 and April 2022, we conducted 21 in-depth interviews with four stakeholder groups representing the EMS response system in Rwanda: ambulance dispatchers, ambulance field staff, receiving hospital staff, and policymakers. Semi-structured interview guides covered participants' perspectives on the challenges EMS systems face in locating an emergency, how challenges impact quality of pre-hospital care, and what opportunities exist for process and tool development. Interviews lasted 30-90 minutes each, and were audio recorded and transcribed. Applied thematic analysis was used to identify themes across three domains: the process of locating an emergency, impacts of challenges, and opportunities for processes and tools. NVivo (version 12) was used to code and organize data.

Results: The current process of locating a patient experiencing an emergency in Kigali is hampered by a lack of supportive technology, depends on knowledge of both the caller and the response team to locate the emergency, and requires multiple calls to share location details between parties (caller, dispatch, ambulance). Three themes emerged related to the impact of challenges in locating an emergency: increased time response, inconsistencies in response based on both the caller and dispatcher staff's individual knowledge of the area, and inefficient communication between the caller, dispatch,

and ambulance. Three themes emerged related to opportunities for processes and tools to improve the location of emergencies: technology to geolocate an emergency accurately and improve time response, improvements in communication to allow for real-time information sharing, and better location data from the public.

Conclusion: This study has identified challenges faced by the EMS system in Kigali, Rwanda in locating emergencies and identified opportunities for intervention. Timely EMS response is essential for optimal clinical outcomes. As EMS systems develop and expand in low-resource settings, there is an urgent need to implement locally relevant solutions to improve the efficient location of emergencies.

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62.   **Biochemical analysis of the Smoothened / Protein Kinase A binding interaction in Hedgehog signaling**

Nathan Iverson

Faculty Mentor: Benjamin Myers (Oncological Sciences, University of Utah)

Abstract

PKA-C complexes is needed to answer these questions, because SMO is the first documented GPCR to directly sequester PKA-C at the membrane, and the first example of a PKA-C decoy substrate whose binding is regulated by

phosphorylation. Here I use several *in vitro* biochemical and structural approaches to address this key knowledge gap. First, I attempted to directly test the role of GRK2/3 phosphorylation in regulating the SMO/PKA-C interaction using binding assays between PKA-C and SMOct engineered to contain mutations that mimic the GRK2/3 phosphorylation events. Second, I prepared a crosslinked complex of SMOct/PKA-C for crystallography studies, thereby overcoming the intrinsically low affinity binding interaction between SMOct and PKA-C that has hampered structural characterization of the complex. Finally, I optimized a protocol for isotopically labeled SMOct preparation in *E. coli* to be used in future NMR studies to overcome the challenges presented by characterizing this largely unstructured domain via traditional crystallography or cryoEM approaches. Understanding the structural and biochemical basis for the SMO-PKA complex will provide critical insights into an essential step in Hh signal transduction and may lead to more effective therapeutic agents to treat a range of different cancers.

INTRODUCTION

The Hedgehog signaling pathway is an essential player in vertebrate embryonic organ development, controlling the formation of nearly every organ in our bodies. This signaling pathway was initially discovered by researchers Nusslein-Volhard and Wieschaus in 1980 from their work in *Drosophila melanogaster* development. The Hh pathway received its name due to the spikey, hedgehog-like appearance of the fruit fly larvae when the Hh gene is knocked out1. Cell proliferation and tissue patterning during development are tuned through Hh ligand gradients that allow for regulated pathway activity in a dose-dependent manner. This promotes cells to adopt specific fates allowing for complex developmental processes

and patterning2. The pathway also has roles in tissue homeostasis and cell maintenance postnatally. Insufficient Hh pathway activity during development results in birth defects or embryonic death. Improper activation postnatally is associated with many cancers including basal cell carcinoma and medulloblastoma. Despite the pathway's importance and association with disease, the signal transduction mechanism has largely remained a mystery.

SMO is a seven-pass transmembrane protein that is the principal determinant of Hh signal transduction at the cell surface. In the pathway "off" state, SMO is inhibited by the transmembrane protein Patched 1 (PTCH1) through an indirect mechanism3. PTCH1 inhibits pathway activity through the transport of cholesterol, which is an endogenous SMO activating ligand4. Researchers have found that PTCH1 depletes cholesterol from the inner leaflet of the plasma membrane rendering the sterol inaccessible to SMO4. This harboring of cholesterol at the outer leaflet of the membrane inhibits SMO activation and expression of target genes.

In the pathway "on" state, Hh ligand binds to PTCH1 which abolishes the transport function of the transmembrane protein, allowing equilibration of cholesterol between the inner and outer leaflet of the plasma membrane. Recent research shows that SMO is activated by a membrane sterol entering a hydrophobic tunnel that connects the inner leaflet to a site in the seven-transmembrane domain of SMO5. The binding of cholesterol induces a conformational change to the protein, initiating the active state of SMO and signaling cascade that leads to the expression of target genes.

Active state SMO initiates activation of the transcription factor GLI through a series of events that was poorly understood until recently. PKA-C is known to be the main

regulator of GLI in the Hh pathway6. In the pathway "off" state, PKA is unrestrained and phosphorylates GLI targeting it by a ubiquitating complex and degraded by the proteasome. When GLI is not phosphorylated by PKA-C, it is an activated transcription factor to promote expression of target genes. We hypothesize that PKA-C activity in the Hh pathway is regulated through direct inhibition by SMO. PKA-C activity is commonly regulated by signal transduction from GPCRs. In canonical GPCR signal transduction, active GPCRs bind to heterotrimeric G proteins at a cytoplasmic domain allowing GTP-bound Gα and free Gβγ to transduce the signal to downstream effector proteins. Typically, GPCRs inhibit PKA-C via coupling to inhibitory heterotrimeric G proteins, which trigger reduction in cytoplasmic levels of cAMP through the membrane protein adenylyl cyclase. cAMP binds to PKA regulatory proteins releasing them from inhibiting PKA-C, and allowing phosphorylation of downstream targets, such as GLI. SMO, however, does not appear to utilize this canonical mechanism to inactivate PKA-C, as both genetic or pharmacological strategies to block inhibitory G proteins do not alter GLI transcriptional activity or expression of Hh pathway target genes7,8. Thus, another pathway mechanism was needed to explain Hh signal transduction.

Arveseth and colleagues uncovered this unconventional mechanism in 2021 finding that SMO directly binds to PKA-C in the active state, harboring the kinase at the membrane and inhibiting its enzymatic activity8,9 (Figure 1). SMO binds to PKA-C via a pseudosubstrate domain present on the cytoplasmic tail of SMO with an amino acid sequence similar to protein kinase inhibitor (PKI)9. PKI is a small, largely unstructured, cytoplasmic protein that binds to the active site of PKA-C with nanomolar affinity. The inhibition of PKA-C is

due to PKI's N-terminal pseudosubstrate domain. This domain binds to the active site of the enzyme independently of cAMP levels and is unable to be phosphorylated, making PKI a potent competitive inhibitor of PKA-C10. The researchers found that SMOct contains a PKI motif in the proximal domain of the tail with four hallmarks that resemble known PKA-C pseudosubstrates. First, the SMOct contains the nonphosphorylatable residue alanine at the canonical phosphorylation site (P site) for PKA-C substrates. The SMOct also contains arginines at the P-2 and P-3 positions that have been shown to be necessary for other pseudosubstrates such as PKI. Finally, SMOct contains a hydrophobic residue at the P+1 site as well as a predicted α-helical domain N-terminal to the pseudosubstrate domain9. These four characteristics are essential in high-affinity binding between PKA-C and pseudosubstrates. These findings suggest a mechanism for how SMOct binds and inhibits PKA-C enzymatic activity at the membrane.

In the pathway "on" state, activated SMO undergoes phosphorylation of the cytosolic tail by GRK2/3 which initiates PKA-C binding. The SMOct/PKA-C binding interaction is likely enhanced when SMOct is phosphorylated by GRK2/3, though this has never been shown directly. Predicted models of the SMOct/PKA-C binding complex suggest a crucial role for phosphorylation of the SMOct in promoting a conformation that will bind to PKA-C. The SMOct/PKA-C binding interaction is relatively weak with an equilibrium dissociation constant (K_D) of 752 nM9. This differs greatly from the PKI/PKA-C binding interaction which has a K_D of 1.1 nM11. Seven GRK2/3 phosphorylation sites present in 3 clusters have been identified on the proximal domain of the SMOct using mass spectrometry8. These sites exhibit dependence on SMO's

activity state and mediate the binding of PKA-C. In addition, two phosphorylation sites are also present on the proximal side of SMOct that are GRK2/3 independent and are weakly associated with SMO activity. Conservation of these 9 phosphorylation sites is seen across many vertebrates suggesting the importance of their role in Hh signaling. When these residues on SMOct involved in GRK2/3 phosphorylation are mutated to alanine, the binding interaction between SMO and PKA-C is greatly diminished8.

This thesis describes experiments to better understand the structural and biochemical basis of the SMOct/PKA-C binding interaction.We hypothesize that GRK2/3 phosphorylation of SMOct will increase the protein's binding affinity to PKA-C through ionic interactions created by the negatively charged phosphate. It is unclear whether phosphorylated SMOct binds directly to PKA-C, or if this phosphorylation promotes binding of an intermediary protein that bridges SMOct and PKA-C. To test these two models using an *in vitro* system, phosphomimetic mutants of SMOct were used in a competition binding assay with PKA-C and compared to wildtype SMOct to determine if phosphorylation decreases the Kd for the SMOct/PKA-C interaction. We utilized phosphomimetic mutation as opposed to GRK2/3 phosphorylation or phosphopeptide synthesis due to the poor ability of GRK2/3 to phosphorylate soluble substrates, and the relative difficulty of phosphopeptide synthesis in comparison to mutation. GRK2/3 phosphorylation of soluble substrates is difficult because GRK2/3 requires binding to the proximal side of the transmembrane domain of the active GPCR, along with membrane lipids (such as PIP2), which then allosterically activates GRK2/3 to phosphorylate the soluble GPCR domains. In the absence of the GPCR's transmembrane domain, such as in SMOct, phosphorylation is

highly inefficient *in vitro* and the turnover number (kcat/Km) decreases 1000-fold when compared with full length

GPCRs. We avoided using full-length SMO due to challenges associated with purifying and solubilizing the membrane protein for binding assays. The SMOct/PKA-C binding interaction is difficult to crystallize for structural analysis due to the weak binding affinity of the two proteins and largely unstructured nature of the SMOct. We tried to overcome this weak binding interaction by crosslinking the SMOct/PKA-C complex to add stability through a covalent bond. We optimized the crosslinking protocol and ran on a large scale to obtain a crosslinked complex that was sent to our collaborators for crystallography.

To obtain structural information about the SMOct peptide, we used NMR as this technique is the only high-resolution atomic-level approach that will allow information about unstructured proteins. NMR has been used in the past to obtain information about unstructured proteins similar to SMOct such as full-length PKI12. I optimized a protocol to express and purify isotopically labeled SMOct to be used in NMR analysis for structural information about the peptide.

Preliminary studies indicate that phosphomimetic mutations did not produce an effect on Kd, which may mean that phosphorylation does not increase binding affinity, though could also reflect technical limitations of the approach I am using. Crosslinking SMOct to PKA-C via a disulfide bond did not produce a crystallizable complex implying that other regions of the protein may need to be stabilized to facilitate crystal formation. These results will help inform ongoing experiments to better understand the SMOct/PKA-C binding interaction and capture this important complex for the first time.

METHODS

SMOct WT and phosphomimetic mutant purification:

SMOct WT and mutant peptides were purified in parallel using a bacterial expression system. An inducible strain of *Escherichia coli*, BL21(DE3), was transduced with the plasmid vector pHTSHP containing SMOct with N-terminal tags MBP-His8-SUMO. The vector pHTSHP also contains an ampicillin resistance gene to allow for selection of transduced cells. Following outgrowth for 1 hour at 37 degrees Celsius in SOC recovery media to allow for expression of ampicillin resistance, the cells were then grown overnight at 37 degrees Celsius in LB containing ampicillin to select for cells expressing the transduced plasmid. 5 mL of the overnight cultures were inoculated into 1 L of LB with ampicillin and grown at 37 deg Celsius until optical density, measured at 600 nm, of 1.0 was reached. Isopropyl β-D-1 thiogalactopyranoside (IPTG) applied to the culture induced the T7 promoter-based expression system to express the SMOct peptide. After induction, the temperature was decreased to 30 degrees Celsius and cells were grown for 5 hours. Cells were then pelleted using centrifugation at 5000 x g for 30 minutes and flash-frozen using liquid nitrogen.

Purification of SMOct from the cell pellet began with resuspension in binding buffer (50mM Tris pH 8, 300 mM NaCl, 25 mM imidazole, 0.2 mM TCEP) followed by cell lysis via sonication in addition to lysozyme and benzonase. The whole cell lysate was clarified via centrifugation and the soluble fraction was isolated. Affinity chromatography using Ni-NTA resin was used to isolate the His8-tagged SMOct peptide. Wash steps used a buffer containing 25 mM imidazole to remove nonspecifically bound proteins on the Ni-NTA resin. Elution of tagged SMOct was performed using 250 mM imidazole and

desired fractions were pooled (Figure 2). Imidazole was then dialyzed from pooled fractions using a two-step dialyzation process. MBP-His8-SUMO tags were cleaved using the protease ULP1 which cleaves the protein at the C-terminal domain of SUMO leaving SMOct untagged. Isolation of untagged SMOct was performed using a three-step process. First, the cleaved solution was applied to Ni-NTA column to isolate untagged SMOct using the buffers described above. The desired fractions were pooled and cation-exchange chromatography was performed to isolate SMOct from remaining impurities. Finally, gel filtration was used to isolate SMOct from SMOct degradation products.

PKA-C purification

Human PKA-C (hPKA-C) and mouse PKA-C (mPKA-C) orthologs were expressed using a bacterial expression system. BL21(DE3)-pLysS cells were transduced with a plasmid containing either hPKA-C (with N-terminal His6-SUMO tags) or mPKA-C (with no tags). The addition of the N-terminal tags improves expression of the protein as it is foreign to *E. coli* and is prone to proteolysis. Following outgrowth for 1 hour at 37 degrees Celsius in SOC recovery media to allow for expression of ampicillin and chloramphenicol resistance, the cells were grown overnight at 37 degrees Celsius in LB containing both antibiotics to select for cells expressing the transduced plasmid as well as the pLysS plasmid. 50 mL of the overnight culture were inoculated into 1 L of LB with both antibiotics and grown at 37 deg Celsius until optical density, measured at 600 nm, of 1.0 was reached. IPTG was used to induce the T7 promoter-based expression system to express the respective PKA-C ortholog. After induction, the temperature was decreased to 18 degrees Celsius and cells were grown overnight. Cells were then pelleted the following day using

centrifugation at 5000 x g for 30 minutes and flash-frozen using liquid nitrogen.

The cell pellet was resuspended in TMN50 buffer (Tris 50 mM pH 7, 50 mM NaCl, 2 mM MgCl2, 0.4 mM ATP) followed by cell lysis via sonication. The lysed cell solution was

clarified using high-speed centrifugation (40,000 x g) for 30 minutes. The clarified sample was incubated with a shortened PKI peptide (IP20) bound to a resin for 90 minutes in binding conditions (400 mM ATP, 1 M MgCl2). IP20 binds to PKA-C with nanomolar affinity and is highly selective to binding PKA-C making it effective in isolating PKA-C from the clarified cell lysate. Incubated sample was applied to a column and washed with TMN50 and TMN250 (Tris 50 mM pH 7, 250 mM NaCl, 2 mM MgCl2, 0.4 mM ATP). PKA-C was then eluted from the IP20 resin using a high arginine buffer to displace the bound PKA-C from the column in 1 mL fractions using Bradford Reagent to monitor when the protein was completely eluted from the column. Fractions were run on an SDS-PAGE gel and PKA-C fractions were pooled.

hPKA-C contains His6-SUMO N-terminal tags that were then cleaved using UPL1 during dialysis to remove arginine from the buffer. mPKA-C is untagged and dialysis was performed in parallel without addition of a protease.

Isotopically labeled SMOct preparation:

Isotopically SMOct was prepared similarly to SMOct grown in LB as described above and only notable differences will be outlined. The overnight growth following transduction was in LB, but minimal media (M9) was used during the outgrowth phase. Minimal media was necessary because it is chemically defined and isotopic labeling can be controlled by simply adding heavy isotopes which is not possible in rich media. The overnight LB culture was diluted into M9 media to an OD

600 of 0.1 and induced with IPTG at OD 600 1.0. Following clarification of the lysed cell pellet, the soluble fraction obtained was boiled to denature the soluble, structured proteins and clarified at 40,000 x g to remove the precipitated proteins. The soluble fraction remaining was purified using Ni-NTA affinity chromatography as described above. Ion exchange and gel filtration was not performed on the sample after Ni-NTA chromatography.

Fluorescence Polarization Assay:

Fluorescence polarization (FP) is a biochemical technique used to quantitatively determine molecular binding interactions. The assay utilizes a fluorophore-bound molecule competing with an untagged molecule for a common binding partner. Polarized light is applied to the sample and excites the fluorophore which emits polarized light proportional to the amount of fluorophore bound to its binding partner which tumbles in solution slowly. When the fluorophore is competed from the binding partner, it rotates in solution rapidly emitting depolarized light when excited by light applied to the sample13. In our case, protein-protein interactions are monitored using a Fluorescin (FAM) tagged, truncated SMOct peptide in competition with untagged SMOct peptide binding with PKA-C (Figure 3). FAM-tagged SMOct and PKA-C were kept at constant concentrations of 40 nM and 3.5 μM respectively. This concentration of FAM-tagged SMOct was chosen to adequately detect changes in polarization while also being low enough for SMOct to compete with the peptide for the PKA-C binding site. The untagged SMOct peptide was serially diluted creating ten SMOct samples with a high concentration of 24 μM to maximally bind the available PKA-C.

The reaction was monitored by measuring polarization using a Tecan plate reader with readings at 0 min, 5 min, 15 min,

and 30 min. Multiple readings were taken at the indicated time points to ensure that the reactions were at equilibrium and to monitor any variation between readings. Each experiment was carried out in at least duplicate with FAM readings at an excitation wavelength of 485 nm and emission wavelength of 535 nm. Using the absorbance data, a dose-response curve was created and IC50 was calculated. This experiment allowed us to compare the binding affinity of SMOct/PKA-C with that of phosphomimetic SMOct/PKA-C.

Crosslinking:

Crosslinking the SMOct/PKA-C complex was employed for structural analysis of the protein-protein binding interaction. SMOct was mutated to contain a cysteine residue at amino acid 637 which is at the P+2 site. Mutation at the P+2 site was chosen for two reasons. First, when this residue was mutated to alanine, binding still occurred between SMOct and PKA-C indicating that mutation to cysteine will not eradicate the binding interaction9. Second, Susan Taylor's lab has mutated the P+2 site on the protein kinase regulatory subunit II to cysteine and crosslinked with a cysteine present in the adjacent binding groove of PKA-C for use in structural studies14. Human PKA-C contains two cysteine residues so to prevent unwanted intramolecular crosslinking, the cysteine at amino acid 343 was mutated to a serine. Purification of the SMOct-(L637C) was carried out as described above for SMOct-WT. Purification of hPKA-C-(C343S) was performed as described above from hPKA-C. Researchers Annabel Lee and Corvin Arveseth optimized the crosslinking on a small scale prior to performing the reaction on a large scale. This included varying the concentration of the two mutant proteins, time, temperature, and pH until efficient crosslinking was observed.

Large-scale crosslinking involved buffer exchange of SMOct-

(L637C) and hPKA-C-(C343S) into a non-oxidizing buffer. Samples were then combined at desired concentrations and incubated for 30 minutes with ATP and MgCl2 to induce SMOct/PKA-C complex. The oxidizing agent diamide was then applied to the sample and incubated for 3 hours to induce cysteine-cysteine crosslink. The product was then run on a non-reducing SDS-PAGE gel to confirm crosslinked product formation.

RESULTS

Phosphomimetic SMOct competition FP assay:

A competition FP assay is able to provide quantitative data in an *in vitro* system. Figure 6 shows the results from a competition FP assay between SMOct-WT and two phosphomimetic SMOct mutants. The SMOct-3D contains mutations to aspartic acid (D) at known GRK2/3 phosphorylation sites within the B cluster (Figure 5). The SMOct-6D mutant contains the same three mutations in the B cluster, but also includes 3 additional aspartic acid mutations at the C cluster.

We hypothesized that these mutations would mimic the effect of a negative charge similar to a phosphate. It is unclear whether the phosphorylated SMOct/PKA-C complex relies on the ionic interactions from having negatively charged phosphates, or if the addition of phosphates provides another role in the protein binding interaction such as steric bulk or an alternate conformation of SMOct. The FP assay provides quantitative data and allows for comparison of binding affinity via the half-maximal inhibitory concentration (IC50). This is a measure of potency and describes what concentration of SMOct is necessary to displace half the fluorescent peptide from the active site of PKA-C. As seen in Figure 6, we do not see a significant difference in IC50 values between the

SMOct-3D mutant and SMOct-WT. This suggests that the presence of negative charges at the B cluster is not sufficient for increased binding affinity between SMOct and PKA-C. It is also possible that an aspartic acid residue does not adequately mimic the size and conformation of a phosphoryl group. The tetrahedral geometry of the phosphoryl group might be necessary in the binding interaction, which would not be well represented by a carboxylate group on an aspartate.

SMOct/PKA-C crosslinking:

Structural analysis of the SMOct/PKA-C complex is difficult to perform due to the low binding affinity between the two proteins, which hinders stable complex formation9. Because of this complication, our understanding of the binding interaction has relied on analogies to existing complexes between PKA-C and high-affinity pseudosubstrates, which are quite different from SMO as explained earlier. To directly characterize the SMO/PKA-C complex in structural terms, we must first overcome the low binding affinity. To this end we employed cysteine crosslinking to covalently link the protein complex for crystallography studies for comparison to predicted models.

Our work focused on the purification and crosslinking of SMOct-(L637C) and hPKA-C-(C343S). As discussed in "Methods," we crosslinked the SMOct mutant to hPKA-C using diamide as the oxidizing agent. Annabel Lee and Isaac Nelson of the Myer's lab optimized the protocol for crosslinking using a small scale binding assay testing various conditions and concentrations of SMOct and PKA-C. We used the optimized protocol on a larger scale to obtain a sufficient sample for crystallography studies. We confirmed the results of the crosslinking using gel electrophoresis (Figure 7). This shows a clear band at 51 kDa that corresponds to the summation of hPKA-C-(C343S) and SMOct-(L637C) which weigh 40 kDa and

11 kDa respectively. We used gel filtration to further purify the crosslinked complex from remaining protein and degradation products. The crosslinked complex was sent to Jessica Bruystens at UCSD for crystallography studies. Several thousand different conditions were tested to crystallize the crosslinked complex and none were successful indicating that there is essentially no way that this complex will crystallize without further increasing the stability.

Isotopically labeled SMOct:

Preparations to optimize the protocol for purification of the isotopically labeled SMOct proved that the expression in minimal media is challenging. Because SMOct is foreign to E. coli, the protein is toxic to the cells and largely inhibits cell growth in minimal media. SMOct expression improved when BL21(DE3) pLysS *E. coli* cells were used to minimize basal expression of SMOct prior to IPTG induction. Even with decreasing basal expression of SMOct, cell growth in M9 media to reach an OD 600 of 1.0 was variable and took 10 to 15 hours which differed greatly from growth in LB where the culture reached the same density in approximately 3 to 5 hours. The yield of SMOct expressed in M9 also decreased compared to LB by a factor of 6. Decreased expression between the LB and M9 media is seen qualitatively in the elution fractions during NiNTA (Figure 8). Thus, preliminary studies demonstrate that expression in the traditional M9 medium is too low to support NMR analysis, and further optimization is needed to purify isotopically labeled SMOct.

DISCUSSION

Phosphomimetic SMOct competition FP assay:

The competition FP binding assay was used to test the hypothesis that phosphorylated SMOct would bind with higher affinity to PKA-C than SMOct without phosphorylation.

Because phosphorylation of the cytoplasmic tail via GRK2/3 requires the seven transmembrane domain of SMO, we were unable to use direct phosphorylation of SMOct and rather mutated known GRK2/3 phosphorylation sites to aspartic acid. Our results indicate that there is no significant change in binding affinity between wildtype SMOct and SMOct-3D, with decreased binding affinity of the SMOct-6D mutant, though the decrease in IC50 from WT is not statistically significant. These results contradict our initial hypothesis and predicted models of the SMOct/PKA-C complex. However, there are possible explanations for this finding. First, the size and shape of the phosphoryl group at the GRK2/3 phosphorylation site could have a role in promoting the correct conformation for SMOct to bind PKA-C. Because the carboxyl group of the aspartic acid side chain is relatively smaller and with a different geometry than a phosphoryl group, it might not mimic the effects of phosphorylation completely. Second, the charge of the phosphoryl group is -1.5 whereas the charge of an aspartate residue is -1.0 at physiologic pH15. This difference in charge between the aspartate residue and phosphoryl group could explain the lack of increased affinity given the importance that the ionic interaction plays in the predicted model. Based on these caveats, the experiments with the phosphomimetic mutations are inconclusive, because we cannot be sure that these mutations accurately reflect the effects of GRK2/3 phosphorylation on the SMOct/PKA-C binding interaction. In addition, mass spectrometry of purified SMO from mammalian cells shows that phosphoryl groups do not necessarily occupy all phosphorylation sites on SMOct at the same time8. It is possible that having three, or six, phosphomimetic mutations on SMOct actually destabilize the conformation required to bind PKA-C. To test this hypothesis in future studies, single

phosphomimetic mutations at the known GRK2/3 phosphorylation sites will be used in the same binding assay to determine if this affects binding with PKA-C.

Additional experimentation to overcome the difficulty with GRK2/3 phosphorylation and complications with phosphomimetics will include synthesis of the SMOct with phosphoserine or phosphothreonine at the GRK2/3 phosphorylation sites. This would effectively overcome all the issues presented by *in vitro* GRK2/3 phosphorylation and phosphomimetic mutation of the SMOct. Because the SMOct is 110 amino acids in length, synthesis of the peptide using solid phase peptide synthesis poses its own set of challenges which is why we did not pursue this route prior to phosphomimetics. However, given the inconclusive nature of our phosphomimetic mutant analysis, we will now attempt to synthesize the phosphopeptide in collaboration with Michael Kay, whose lab has extensive expertise with synthetic peptide synthesis. The synthetic phosphopeptide could then be used in a binding assay with PKA-C and compared to WT SMOct to determine the effects of phosphorylation on SMOct binding affinity.

Another possible experiment to overcome the difficulties presented is to phosphorylate full length SMO in detergent micelles directly with GRK2/3 in the presence of the membrane lipid PIP2 (which allosterically activates GRK2/3, see Introduction). This approach allows GRK2/3 to dock to the intracellular domain of SMO and the membrane lipid PIP2 to then phosphorylate SMOct which is not possible with soluble substrates such as SMOct. Other members of the Myers lab are currently working on a protocol for *in vitro* phosphorylation of SMO which could then potentially be used in a similar binding assay to determine the effects of direct phosphorylation in binding with PKA-C.

SMOct/PKA-C structural analysis:

Structural analysis of the SMOct/PKA-C complex proves to be difficult via conventional structural biology methods. This is due to the transient nature of the binding interaction which makes techniques such as crystallography and cryogenic electron microscopy difficult. We hypothesized that covalently crosslinking the complex via a disulfide bond would stabilize the two proteins and enable crystallization. My work focused on the preparation of the protein complex which was sent to Susan Taylor's lab at UCSD for crystallography. Despite modification to covalently bond the two proteins, the SMOct/PKA-C complex did not crystallize and we were unable to obtain structural information from this experiment. This result suggests that the protein complex requires further stabilization to crystallize. Possible ways to stabilize the complex include additional covalent bond attachments, truncation of the SMOct peptide to remove unstable regions, or addition of antibodies/nanobodies that support the bound complex.

Another route I am exploring to obtain structural information regarding SMOct is through NMR due to the ability to obtain information about unstructured proteins at an atomic level. Structural information about the binding interaction of SMOct/PKA-C can be observed through chemical shift perturbations in the NMR amide fingerprint of isotopically labeled SMOct. This technique was used to pinpoint the residues on PKA-C that are involved in binding to SMOct, though it has not been performed with isotopically labeled SMOct.

My work is currently focused on optimizing a protocol to express isotopically labeled SMOct in *E. coli*. Minimal media is required to grow the cells to ensure the culture only contains isotopically labeled nitrogen (15N). Expression levels of SMOct

in minimal media continue to remain low despite attempts to decrease basal protein expression prior to induction. This is likely due to a combination of factors including SMOct toxicity to *E. coli* as well as decreased rate of bacterial growth in minimal media. One possible method to overcome this difficulty in expression is through autoinduction. This method of bacterial protein expression utilizes a culture medium containing both glucose and lactose. Glucose is preferentially used as an energy source by *E. coli*, but once the glucose in the media is consumed, lactose is then metabolized producing allolactose and activating expression of target genes via the *lac* operon. This protocol offers advantages over IPTG induction for toxic proteins as the bacteria are able to self-regulate protein expression levels. This allows for gradual protein expression that can prevent the deleterious effects of toxic proteins on bacterial growth16. This method is encouraging for growth of isotopically labeled SMOct given its success in the past with SMOct truncated peptides and with other recombinant proteins that are toxic to bacteria.

Ciliary regulation of SMO:

To explain why the SMOct/PKA-C binding interaction is a low affinity interaction and relevant in PKA-C sequestration by SMO, physiologic conditions have to be considered. The primary cilium is essential for Hh signal transduction17. This is due to the compartmentalization of SMO which, when activated, accumulates 20-fold in the primary cilium18. This increase in the local concentration of SMO allows for regulation of a smaller pool of ciliary PKA-C, as most cytoplasmic PKA-C is inactive and bound in its holoenzyme form with protein kinase A regulatory subunits (PKA-R). This is an important regulatory mechanism of the Hh pathway as PKA is used in myriad cellular processes19. SMOct does not

bind to the PKA holoenzyme8, as binding is specific to the PKA-C active site which is not available when PKA-R is bound. This compartmental regulation of PKA-C is physiologically important as it allows Hh pathway activation to target GLI without disrupting PKA-dependent processes throughout the cell20. It also offers insight into how the SMO/PKA-C interaction, while of only modest affinity, may nevertheless be highly relevant during Hh signal transduction in its native ciliary environment.

Because SMO is acting on a smaller, defined pool of ciliary PKA-C, small changes in free PKA-C concentration have the potential to create large effects given that gene expression is the final target of the Hh pathway. Continuing to improve understanding of this low affinity interaction between SMOct and PKA-C through biochemical and structural studies will be vital to understanding the complete mechanistic details of Hh signal transduction.
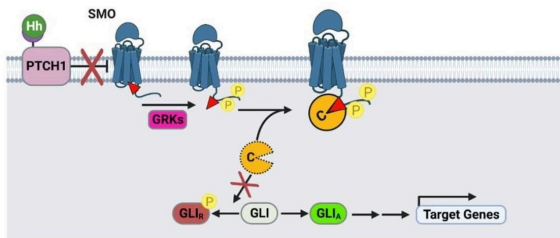
Figure 1: Diagram of the Hh pathway "on" state highlighting GRK phosphorylation of the SMOct which promotes PKA-C sequestration and activation of downstream target genes via active GLI. Red triangle on the SMOct represents the PKI motif
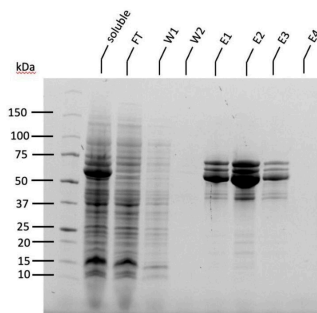
Figure 2: SDS-PAGE gel following initial NiNTA purification of His-tagged SMOct from E. coli. The "soluble" lane is a sample of the whole cell lysate following clarification. "FT" indicates the flow through from the sample that was applied to the NiNTA column. "W1" and "W2" are the wash steps in low imidazole buffer. "E1", "E2", "E3", and "E4" indicate the respective elutions in high imidazole buffer. SMOct expressed with MBP, His, and SUMO tags is expected to have a weight of 72.2 kDa.
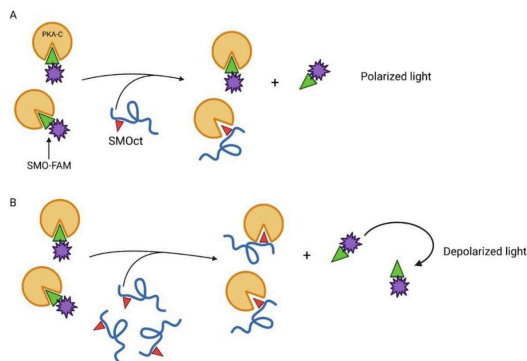
Figure 3: Diagram of the competition FP assay with SMOct competing with the SMO-FAM peptide for PKA-C. A) SMOct is in low concentration leaving a high proportion of SMO-FAM peptide bound to PKA-C. When SMO-FAM is bound to PKA-C, polarized light is detected. B) SMOct is in high concentration leaving a relatively low proportion of SMO-FAM peptide bound to PKA-C. SMO-FAM now rotates freely in solution which produces depolarized light.
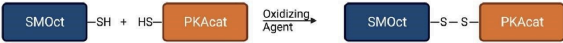
Figure 4: Diagram showing the crosslinking of mutated SMOct to PKA-C. Cysteine residues on the mutated SMOct and PKA-C were exposed to the oxidizing agent diamide to covalently link the proteins in a disulfide bond.
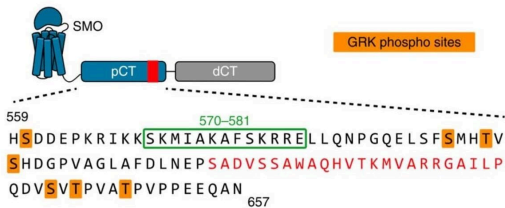
Figure 5: Diagram of SMO highlighting the residues and GRK2/3 phosphorylation sites of the proximal cytoplasmic tail (pCT). Residues boxed in green indicate a predicted alpha-helical region of the pCT. Residues in red indicate the PKI motif. Orange highlighted residues 593, 596, and 598 make up the B cluster. Orange highlighted residues 642, 644, and 648 make up the C cluster.
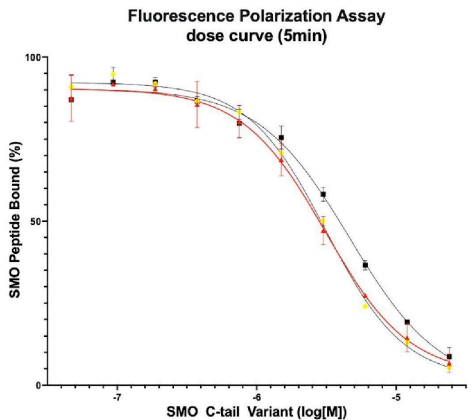
Figure 6: Results from competition FP assay comparing the binding affinity of WT SMOct to SMOct phosphomimetic mutants. WT SMOct is shown in red with an IC50 of 3.12 μM +/- 0.56 μM. SMOct-3D and SMOct-6D are shown in yellow and black respectively. IC50 for SMOct-3D is equal to 3.14 μM +/- 0.60 μM and SMOct-6D is 4.64 μM +/- 1.1 μM.
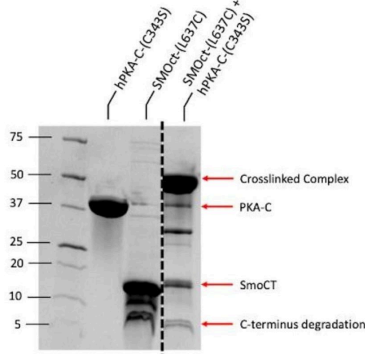
Figure 7: Non-reducing SDS-PAGE gel confirming crosslinking of SMOct-(L637C) and hPKA-C-(C343S). All lanes are from the same gel, though have been spliced for clarity (indicated by dashed line). Lane 1 shows an annotated protein ladder. Lane 2 shows purified hPKA-C-(C343S) with a molecular weight of 40 kDa. Lane 3 shows purified SMOct-(L637C) with a molecular weight of 11 kDa. Lane 4 shows formation of the crosslinked product following incubation of SMOct-(L637C) and hPKA-C-(C343S) in the presence of diamide.
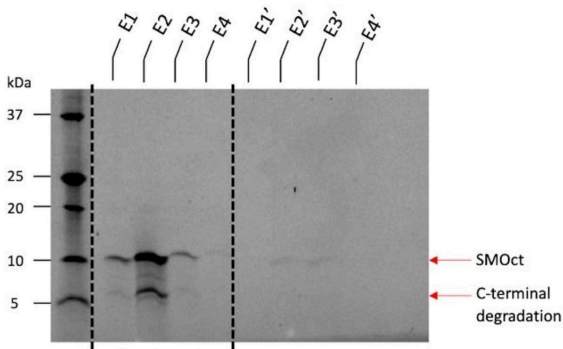
Figure 8: SDS-PAGE gel comparing NiNTA elutions from LB and M9 culture media. All lanes are from the same gel, though have been spliced for clarity (indicated by dashed lines). E1 through E4 are the respective elutions in LB. E1' through E4' are the respective elutions in M9.

REFERENCES

(1) Nüsslein-Volhard, C.; Wieschaus, E. Mutations Affecting Segment Number and Polarity in Drosophila. *Nature* **1980**, *287* (5785), 795–801. https://doi.org/10.1038/287795a0.

(2) Pak, E.; Segal, R. A. Hedgehog Signal Transduction: Key Players, Oncogenic Drivers, and Cancer Therapy. *Dev. Cell* **2016**, *38* (4), 333–344. https://doi.org/10.1016/j.devcel.2016.07.026.

(3) Taipale, J.; Cooper, M. K.; Maiti, T.; Beachy, P. A. Patched Acts Catalytically to Suppress the Activity of Smoothened. *Nature* **2002**, *418* (6900), 892–897. https://doi.org/10.1038/nature00989.

(4) Zhang, Y.; Bulkley, D. P.; Xin, Y.; Roberts, K. J.; Asarnow, D. E.; Sharma, A.; Myers, B. R.; Cho, W.; Cheng, Y.; Beachy, P. A. Structural Basis for Cholesterol Transport-like Activity of the Hedgehog Receptor Patched. *Cell* **2018**, *175* (5), 1352-1364.e14. https://doi.org/10.1016/j.cell.2018.10.026.

(5) Deshpande, I.; Liang, J.; Hedeen, D.; Roberts, K. J.; Zhang, Y.; Ha, B.; Latorraca, N. R.; Faust, B.; Dror, R. O.; Beachy, P. A.; Myers, B. R.; Manglik, A. Smoothened Stimulation by Membrane Sterols Drives Hedgehog Pathway Activity. *Nature* **2019**, *571* (7764), 284–288. https://doi.org/10.1038/s41586-019-1355-4.

(6) Niewiadomski, P.; Kong, J. H.; Ahrends, R.; Ma, Y.; Humke, E. W.; Khan, S.; Teruel, M. N.; Novitch, B. G.; Rohatgi, R. Gli Protein Activity Is Controlled by Multisite Phosphorylation in Vertebrate Hedgehog Signaling. *Cell Rep.* **2014**, *6* (1), 168–181. https://doi.org/10.1016/j.celrep.2013.12.003.

(7) Low, W.-C.; Wang, C.; Pan, Y.; Huang, X.-Y.; Chen, J. K.; Wang, B. The Decoupling of Smoothened from Galphai Proteins Has Little Effect on Gli3 Protein Processing and Hedgehog-Regulated Chick Neural Tube Patterning. *Dev. Biol.* **2008**, *321* (1), 188–196. https://doi.org/10.1016/j.ydbio.2008.06.014.

(8) Arveseth, C. D.; Happ, J. T.; Hedeen, D. S.; Zhu, J.-F.; Capener, J. L.; Shaw, D. K.; Deshpande, I.; Liang, J.; Xu, J.; Stubben, S. L.; Nelson, I. B.; Walker, M. F.; Kawakami, K.; Inoue, A.; Krogan, N. J.; Grunwald, D. J.; Hüttenhain, R.; Manglik, A.; Myers, B. R. Smoothened Transduces Hedgehog Signals via Activity-Dependent Sequestration of PKA Catalytic Subunits. *PLOS Biol.* **2021**, *19* (4), e3001191. https://doi.org/10.1371/journal.pbio.3001191.

(9) Happ, J. T.; Arveseth, C. D.; Bruystens, J.; Bertinetti, D.; Nelson, I. B.; Olivieri, C.; Zhang, J.; Hedeen, D. S.; Zhu, J.-F.; Capener, J. L.; Bröckel, J. W.; Vu, L.; King, C. C.; Ruiz-Perez, V. L.; Ge, X.; Veglia, G.; Herberg, F. W.; Taylor, S. S.; Myers, B. R. A PKA Inhibitor Motif within SMOOTHENED Controls

Hedgehog Signal Transduction. *Nat. Struct. Mol. Biol.* **2022**, *29* (10), 990–999. https://doi.org/10.1038/s41594-022-00838-z.

(10) Liu, C.; Ke, P.; Zhang, J.; Zhang, X.; Chen, X. Protein Kinase Inhibitor Peptide as a Tool to Specifically Inhibit Protein Kinase A. *Front. Physiol.* **2020**, *11*, 574030. https://doi.org/10.3389/fphys.2020.574030.

(11) Knape, M. J.; Ballez, M.; Burghardt, N. C.; Zimmermann, B.; Bertinetti, D.; Kornev, A. P.; Herberg, F. W. Divalent Metal Ions Control Activity and Inhibition of Protein Kinases. *Metallomics* **2017**, *9* (11), 1576–1584. https://doi.org/10.1039/c7mt00204a.

(12) Olivieri, C.; Wang, Y.; Li, G. C.; V S, M.; Kim, J.; Stultz, B. R.; Neibergall, M.; Porcelli, F.; Muretta, J. M.; Thomas, D. D.; Gao, J.; Blumenthal, D. K.; Taylor, S. S.; Veglia, G. Multi-State Recognition Pathway of the Intrinsically Disordered Protein Kinase Inhibitor by Protein Kinase A. *eLife* **2020**, *9*, e55607. https://doi.org/10.7554/eLife.55607.

(13) Moerke, N. J. Fluorescence Polarization (FP) Assays for Monitoring Peptide-Protein or Nucleic Acid-Protein Binding. *Curr. Protoc. Chem. Biol.* **2009**, *1* (1), 1–15. https://doi.org/10.1002/9780470559277.ch090102.

(14) First, E. A.; Taylor, S. S. Induced Interchain Disulfide Bonding in CAMP-Dependent Protein Kinase II. *J. Biol. Chem.* **1984**, *259* (7), 4011–4014.

(15) Dephoure, N.; Gould, K. L.; Gygi, S. P.; Kellogg, D. R. Mapping and Analysis of Phosphorylation Sites: A Quick Guide for Cell Biologists. *Mol. Biol. Cell* **2013**, *24* (5), 535–542. https://doi.org/10.1091/mbc.E12-09-0677.

(16) Blommel, P. G.; Becker, K. J.; Duvnjak, P.; Fox, B. G. Enhanced Bacterial Protein Expression During Auto-Induction Obtained by Alteration of Lac Repressor Dosage and Medium

Composition. *Biotechnol. Prog.* **2007**, *23* (3), 585–598. https://doi.org/10.1021/bp070011x.

(17) Gigante, E. D.; Caspary, T. Signaling in the Primary Cilium through the Lens of the Hedgehog Pathway. *Wiley Interdiscip. Rev. Dev. Biol.* **2020**, *9* (6), e377. https://doi.org/10.1002/wdev.377.

(18) Corbit, K. C.; Aanstad, P.; Singla, V.; Norman, A. R.; Stainier, D. Y. R.; Reiter, J. F. Vertebrate Smoothened Functions at the Primary Cilium. *Nature* **2005**, *437* (7061), 1018–1021. https://doi.org/10.1038/nature04117.

(19) Bangs, F.; Anderson, K. V. Primary Cilia and Mammalian Hedgehog Signaling. *Cold Spring Harb. Perspect. Biol.* **2017**, *9* (5), a028175. https://doi.org/10.1101/cshperspect.a028175.

(20) Tuson, M.; He, M.; Anderson, K. V. Protein Kinase A Acts at the Basal Body of the Primary Cilium to Prevent Gli2 Activation and Ventralization of the Mouse Neural Tube. *Development* **2011**, *138* (22), 4921–4930. https://doi.org/10.1242/dev.070805.

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63. Treatment Prediction of Depression from a Neuro-Motor Response Perspective

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There are over 350 million people suffering from major depressive disorder (MDD) worldwide. Roughly half of all these patients are resistant to first line antidepressants [2]. While nearly all existing research in MDD has focused on cognitive and emotional domains, the research being conducted at

University of Utah research park is investigating depression amongst individuals motor function. With the data collected from the investigation we can find what treatment is best for the individual going further into diagnosis.The lab has hypothesized that depression amongst adults affects their motor skills overtime and thus will lead to better approaches to treatment for mental disorders. Depression is a common and serious medical illness that negatively affects mood, and one's actions. "Depression causes feelings of sadness and/or a loss of interest in activities you once enjoyed. It can lead to a variety of emotional and physical problems and can decrease your ability to function at work and at home" [4]. The interplay of body and mind seems relevant during the development of cognitive decline and dementia. The measurement of gait speed may improve the detection of prodromal dementia and cognitive impairment in individuals with and without initial cognitive deficits [1]. We propose to build models based on motor composite scores that reflect performance across all motor domains. Based on our pilot data of with MDD and control subjects, we want to detect a significant difference in the following motor measures: 1) grip strength of the dominant and non dominant hand; 2) spiral tracing, 3) 4-meter walk test; and 4) errors made during the walking while-talking test. In order to test whether these variables are significant, we will furthermore analyze if motor behavioral and neural measures measured while depressed patients are in their treatment phase are predictive of time-to-remission using statistical analysis. As for our future research, by integrating such neurobiological measures (MRI), we will be able to gain important insight into MDD etiology and the role of motor dysfunction in MDD [3]. This will also enable us to develop a better understanding as to whether motor dysfunction among various MDD subtypes is

transient, permanent, and the degree to which it can be used as a valid biomarker.

References:

[1] Grande G, Triolo F, Nuara A, Welmer AK, Fratiglioni L, Vetrano DL. Measuring gait speed to better identify prodromal dementia. Exp Gerontol. 2019 Sep;124:110625. doi: 10.1016/j.exger.2019.05.014. Epub 2019 Jun 4. PMID: 31173841

[2] "Major Depression." National Institute of Mental Health, U.S. Department of Health and Human Services, https://www.nimh.nih.gov/health/statistics/major-depression.

[3] "Depression (Major Depressive Disorder)." Mayo Clinic, Mayo Foundation for Medical Education and Research, 3 Feb. 2018, https://www.mayoclinic.org/diseases-conditions/depression/diagnosis-treatment/drc-20356013.

[4] American Psychiatric Association. "What Is Depression?" What Is Depression?, https:// www.psychiatry.org/patients-families/depression/what-is-depression.

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64. **Research Reflection by Jasmine Jacobo**

Jasmine Jacobo

Undergraduate research has always been daunting for someone who is not experienced in the field, especially coming from a first-generation perspective it was hard to navigate. With help from my mentors, I have found a new love for research. I have been able to work with patients who I get to not only one day help, but also learn from. I've been able to learn so many different skills such as data analytics, 3D printing, and patient handling skills. My future goals are to attend medical school and specialize in the field of psychiatry. This had been an incredible experience by giving me hands-on experience working with individuals that are struggling with similar mental illnesses. The impact it has had on me is one I will take with me forever. I'm no longer intimidated by the idea of research, rather I'm more excited than ever when there is a challenge waiting for me. There is so much further research to do, despite the long haul, I'm so grateful to have

had this experience working at the lab and with my mentors that have taught me so much along the way.

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65. Upward Effortful Pitch Glide as a Predictor of Swallow Function in Upper Vs. Lower Motor Neuron Lesions

Scott C. King; Julie M. Barkmeier-Kraemer (Otolaryngology); Elizabeth Lanza; Benjamin Schiedermayer; and Jennifer Herrick (Internal Medicine)

Faculty Mentor: Julie Barkmeier-Kraemer (Otolaryngology, University of Utah)

Objective: This study addressed the correspondence between maximum pharyngeal constriction (PCmax) during effortful upward pitch glide task using nasoendoscopy compared with similar measures from videofluoroscopic swallow studies (VFSS) of swallowing function in individuals with upper (UMN) vs lower motor neuron (LMN) lesions. Whereas videofluoroscopy enables measurement of the timing and distance of structural movements during swallowing (a non-volitional task), nasoendoscopy enables direct observation of the upper airway structures and evaluation of pharyngeal function during a volitional vocal task considered predictive of swallow function. The purpose of this study was to evaluate the use of a volitional vocal task during nasoendoscopy to predict non-volitional pharyngeal function during swallowing in patients with upper vs lower motor neuron lesions.

Methods: Comparisons were made between PCmax measures from VFSS and nasoendoscopy in 26 individuals with UMN (N = 13) or LMN (N = 13) etiologies of dysphagia. Two judges rated the degree and symmetry of PCmax in paired nasoendoscopic images contrasting quiet breathing (i.e. minimum pharyngeal constriction) and highest pitch production (i.e. PCmax). Judges rated each subject twice in a randomized order to obtain intra- and inter-rater reliability estimates. Judge 1 achieved 96% and Judge 2 achieved 92% intra-rater agreement. Inter-rater agreement was 63% overall. Judges reached consensus on items with differing ratings prior to final analysis. Final nasoendoscopy PCmax ratings were compared to VFSS measures within and between UMN and LMN groups using univariate linear regression analysis using an alpha level of .1 due to the small sample size.

Preliminary Results: A significant difference occurred

between nasoendoscopic ratings and VFSS PCmax measures between UMN and LMN groups (p<.054). Stronger correspondence was shown for the LMN group than the UMN group. Nasoendoscopic ratings did not accurately predict VFSS PCmax measurement outcomes in 1/3 of the UMN group.

Conclusions: Nasoendoscopic ratings of PCmax during upward pitch glide may not accurately predict VFSS measures in 1/3 of individuals with UMN lesions.

Discussion: The UMN group was primarily comprised of subjects with subcortical lesions. For this reason, in addition to a small initial sample size, this study will continue until we can recruit 20 participants in the LMN group, and 10 subcortical and 10 cortical participants in the UMN group. Data analysis is anticipated to be complete in February 2023.

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66.   **An examination of the Relationship Between Positive COVID-19 Infection and Vaccine Hesitancy Within the Utah Recover Study Population**

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ABSTRACT

Vaccine hesitancy is defined by the World Health Organization as a "delay in acceptance or refusal of vaccines despite availability of vaccination services," and is affected by a wide range of factors. Some of these factors identified in the literature include perceived safety and importance of vaccines, as well as high levels of fear regarding the disease in question. This study utilized data from the Utah RECOVER Study (Research on the Epidemiology of SARS-CoV- 2 in Essential Response Personnel), which is a prospective cohort study, to evaluate whether participants' perception regarding chances of future infection, anxiety regarding future infection, and the importance of obtaining COVID-19 immunizations is changed after being diagnosed with an infection. Using a difference-in-difference calculation, participants who were identified as being COVID-19 positive during the duration of the study did not experience as great an increase in their concern for future COVID-19 infections as did the uninfected group. Furthermore, their concern and anxiety regarding future infections trended towards not increasing as much as those who did not have an infection. By understanding how a history of infection with a particular disease may impact perceptions of future risk and vaccination, approaches toward public health education and campaigns can be modified.

INTRODUCTION

Vaccine hesitancy is defined by the World Health Organization as a "delay in acceptance or refusal of vaccines despite availability of vaccination services," and is largely affected by a wide range of factors (Jasarevic, 2015). When

the COVID-19 vaccine was first introduced in August 2020, herd immunity was an initial public health focus to reduce the impact of the spread of SARS-CoV-2. While the explicit percentage of the population needed to achieve the early goal of herd immunity was not clearly identified, comparisons to diseases such as measles suggested that it would require up to 95% of the population to become vaccinated to reach this goal (Wilder-Smith, 2021).

To date, there have now been over 976 million vaccines administered in the United States, with over 81.4% percent of our entire population now having received at least one dose of the vaccination and only 69.4% having completed a primary series of vaccinations (Centers, 2023b). These vaccination rates fall well short of the early stated immunization goals associated with what was felt to be an opportunity for herd immunity; ultimately, additional investigations in 2022 indicated that herd immunity may not be plausible due to the potential instability of the vaccine and the resulting inability to offer long-term protection to vaccine recipients (Morens, 2022). However, the gap in vaccine uptake despite the far-reaching public health efforts during the early pandemic indicates that vaccine hesitancy was prevalent (World, 2020; Wilder-Smith, 2021).

The Centers for Disease Control has identified at least twelve separate variants that have existed at varying times during the past three years, suggesting that additional variants may continue to emerge (Centers, 2023a). Thus, while recent COVID-19 case numbers and deaths have continued to decline, this ongoing resistance or hesitancy to becoming vaccinated is an ongoing concern for improving the rate of COVID-19 vaccinations (Sekizawa, 2022).

Current literature suggests that safety and importance are major concerns with regard to the reception of vaccines. A

study completed in July-August 2020 indicated that 36% of the studied population were reluctant to receive a COVID-19 vaccination, stating that the most frequent reason offered was a "lack of confidence in the safety of the vaccines, followed by lack of confidence in the effectiveness of the vaccine" (Wiysonge, 2022). Concerns regarding safety and importance are not specific to the COVID-19 vaccine, as other vaccines have had hesitancy associated with these issues, however, with regards to COVID-19, this may have been compounded by what has been referred to as the "Covid Infodemic" (Dubé, 2022) or the intensive modern spread of large amounts of information or misinformation that have created doubt about whether a novel vaccine is safe or important, despite the availability of the service.

While the importance of the vaccine, as well as the safety of immunization are significant issues, it is necessary to acknowledge that other factors have been identified as playing a role in vaccine hesitancy during the pandemic. This includes concerns surrounding the efficacy of the COVID-19 infection and/or vaccine, the current dissemination of information in this digital age, socioeconomic status, political climate and/or personal status, and religious and cultural beliefs (Fiselmann, 2022). When delving into the specific topic of infection, the Sekizawa study compared the potential associations between fear of COVID-19 and COVID-19 hesitancy, concluding that those in their study population that identified as having high levels of fear concerning COVID-19 were more willing to receive a vaccine a year later into the study. This result could be attributed to the fact that vaccination is an action that an individual fearful of the virus will take to address their fear.

The Sekizawa study also broke down the fear of COVID-19 into multiple components: a fear of how novel current

knowledge is of the virus, how effective or safe the available vaccines actually are, how beneficial currently enacted health measures have been in infection prevention, and a fear of how severe viral infection could be. But the most notable worry is the common concern of a fear of actually becoming infected with the virus (Sekizawa, 2022). Given that fear of being infected appears to be a main driver in leading people to overcome their vaccine hesitancy, another aspect to understand is what, if any, the effect that a person's own infection has on their views of the vaccine.

Prior infection with COVID-19 has been documented to offer a high level of protection against future reinfection. Various randomized placebo-controlled trials found that COVID-19 vaccinations had a range of 66% to 95% effectiveness in preventing symptomatic COVID-19 infections, a range similar to the 87% protection offered by native immunity. (Pooley, 2023). However, Pooley also found that immunity from both forms of immunity, native or vaccination, wanes over time. This was also illustrated in an additional study conducted in August 2021 that examined a population in Kentucky to compare the likelihood of reinfection between a group who had already been previously infected with COVID-19 and declined vaccination and those who were fully vaccinated. The article found that the unvaccinated, previously infected group, was two times as likely to become reinfected in comparison to those who were fully vaccinated (Cavanaugh, 2021).

Despite such studies suggesting native immunity to be imperfect, other research indicated that a prior COVID-19 infection resulted in a 50% decrease in a participant's likelihood of receiving a subsequent vaccine (Do, 2022). While prior infection is only one of many diverse variables that can cause an individual to experience vaccine hesitancy, the association

between prior infection and vaccine hesitancy is an important subject to investigate as understanding how to improve vaccine uptake in a post-infected population may be critical in protecting from reinfection and subsequent sequelae when native immunity is suboptimal.

In this study, we consider what effect infection with SARS-CoV2 has on participants' perception regarding the likelihood of future infections, concern regarding future infection, and ultimately, the importance of COVID vaccination. Our hypothesis states that participants who have had at least one positive COVID-19 nasal swab PCR test in the past 9 months are more likely to have a positive perception of vaccinations. While previous literature has stated that there is contrary evidence to this hypothesis, our study collects data beyond the timeframe of prior studies, after additional variants and their resultant spikes of infection occurred. Additionally, with our population being public-facing, essential workers, vaccination would be one of the more important and accessible options for participants to protect themselves against acquiring infection.

METHODS

AN INTRODUCTION TO THE RECOVER STUDY

The RECOVER study is a program funded by the United States Centers for Disease Control (CDC). This program, which was originally initiated in August 2020, is in current collaboration with six different study sites across the United States. These study sites are the University of Miami, in Miami, Florida; St. Luke's Hospital in Duluth, Minnesota; Baylor Scott and White Health in Temple, Texas; University of Arizona in Tuscon, Arizona; Kaiser Permanente Northwest in Portland, OR; and University of Utah in Salt Lake City, Utah. While the CDC is the source of funding for the project, the RECOVER study design was developed by the CDC in conjunction with

Abt Associates, as well as input from investigators at each of the above-listed study sites.

The RECOVER study was initially designed to enroll approximately 3000 essential workers across the 6 study sites and to follow them through the use of active surveillance for at least 18 months. The CDC, Abt Associates, and the investigators at the study sites decided on a number of primary and secondary objectives that ranged from vaccine effectiveness, frequency of SARS-CoV-2 infection and COVID-19 illness, to characterizing the knowledge, attitudes, and practices related to vaccines, and questions regarding immunogenicity among many other questions.

At the start of the study and throughout, medical history, vaccination documentation, and participant demographics were gathered. All participants involved with this study provided online informed consent to indicate their participation. The study protocol and procedures were evaluated and approved by 5 separate IRBs, including the University of Utah. All methods utilized by the study were carried out in observance of current regulations and guidelines.

INCLUSION/EXCLUSION CRITERIA

The eligibility criteria for the RECOVER study consisted of individuals who were either healthcare personnel or frontline workers that had essential jobs that could not be performed from home. One key definition of these essential workers was that they worked at least twenty hours per week and had "direct face-to-face contact, defined as being within 3 feet, or about arm's length, with co-workers, patients or the public as part of job responsibilities" (University, 2023). An Eligibility Screening Interview was conducted with interested participants to determine those who were eligible. Exclusion criteria included those who do not meet the definition of

essential workers, already received a COVID-19 vaccine or had participated in a COVID-19 prevention or treatment investigational trial in the 3 months prior to screening for the RECOVER study.

Specific to this study, participants were selected who had enrolled at the University of Utah site, and answered "No" to the question "Were any of the test results positive or confirmed you were infected?" Infection in the context of the question was the COVID-19 virus. Participants who answered "Yes" to this question were excluded to isolate only the responses of individuals who have never been infected with this particular virus. Participants who did not open the survey or did not provide an answer to the above question were excluded from the data set.

RECRUITMENT

Recruitment was undertaken in phases, with healthcare workers and first responders being the initial group. Later phases included the recruitment of essential frontline workers. Efforts were made to recruit a specific number of participants per occupation, age, and sex stratum at each site. Each site had its own incentive structure; at the University of Utah, participants would receive various amounts of monetary incentives for completing various assigned tasks such as submitting weekly nasal swabs, completing blood draws, or committing to the research study for a year. When the initial study period was coming to a close, the study activities were extended; currently, July 31st, 2023 will be the last day of active participant surveillance and will mark the end of raw data collection for the RECOVER study as a whole.

PARTICIPANT DATA COLLECTION & QUESTIONNAIRE CREATION

The RECOVER study gathers data through three different

collection methods: electronic surveys, mid-turbinate nasal swab collections, and blood samples. Text messages, access to medical reports, and emails are used to perform active surveillance. Surveys are sent at various intervals to assess general participant health, usage of personal protective equipment (PPE), frequency of direct contact with other individuals, as well as potential COVID-19 symptoms related to infection or vaccination. Depending upon the survey content, the surveys may be sent weekly, every three months, or after testing positive for COVID-19. Results are collected and organized by RedCap, an online platform that manages online databases and surveys.

In addition to the electronic surveys, on a weekly basis, participants complete at-home, mid-turbinate nasal swab kits. If the participant is having predefined symptoms suggestive of a COVID-illness, an additional anterior nasal dry foam swab, and a saliva sample are also collected. The participants receive assembled kits on a periodic basis from the RECOVER study group, and the completed swabs were sent back to the RECOVER office where research assistants scan and ship the samples directly to a CDC laboratory for reverse transcription polymerase chain reaction (RT-PCR) analysis. Participants are notified of the results of these samples by email on a weekly basis.

Finally, blood samples were collected at enrollment and every 3 months thereafter; additional samples were gathered 28 days after confirmed COVID-19 infection and 14-28 days after a COVID-19 dose. For the University of Utah site, the Clinical & Translational Science Institute (CTSI) assisted our on-site clinical research staff in collecting blood specimens.

Much of the data collected for the broader RECOVER study is not directly relevant to answer the current questions. For this

particular examination, surveys sent out every three months from March 31st, 2021 to December 16th, 2022 were utilized for data analysis. This timeframe includes six surveys in total; four of these surveys were used to narrow the amount of analyzed data (Survey 2, Survey 4, Survey 6, and Survey 7); and for the statistical analysis, the earliest and latest survey were used in the calculation. Per each three-month survey, the Utah RECOVER Study sent out 1,181 surveys; Survey 2 received 720 responses, Survey 4 received 480 responses, Survey 6 received 612 responses, and Survey 7 received 605 responses. The specific time periods for each survey are as follows: Survey 2 (March 31, 2021-June 22nd, 2021), Survey 4 (November 17th, 2021-February 15th, 2022), Survey 6 (June 30th, 2022-September 18th, 2022), and Survey 7 (September 22nd, 2022-December 16th, 2022).

Data from nasal PCR swab samples were utilized as opposed to blood serum results. This is secondary to the fact that the PCR swab sample results had a quick turnaround time of 3-5 days in comparison to the delayed response of 3-6 months from blood serum results. Such a delay was felt to make capturing the effect of blood serum results on a participant's willingness to receive the vaccine more difficult than utilizing the more readily available nasal swab results that participants were asked to complete each week.

OUTCOME VARIABLES

The outcome variables of interest from the above-described surveys were the questions seeking to identify participants' perceived risk of acquiring COVID-19, anxiety or concern with acquiring COVID-19, and the importance of receiving the COVID-19 vaccine. Specifically, the three separate questions examined were phrased as follows: 1. "Think ahead to the next

6 months. What do you think your chances are of becoming infected or ill with COVID-19?"

1. This question offers a scale of values "0"-"6" or "2"-"8", with the range of potential answers being almost zero chance, very small chance, small chance, moderate chance, large chance, very large chance, and almost certain. The two different scales of value were a result of different coding designations that occurred during the creation of Survey 2 versus Survey 4. The difference in numerical value was adjusted to account for the same answer range across the four surveys. (It will be identified moving forward as "CovidChance", which is a reference to the codebook name it was originally designated as during the creation of the survey.)

2. "Think ahead to the next 6 months. How worried are you about becoming infected or ill with COVID-19?"

1. This question offers a scale of values "0"-"4", with the answer range being not at all worried, a little worried, moderately worried, very worried, and extremely worried. (It will be identified as "CovidWord".)

3. "How important do you think getting an additional COVID-19 vaccine or booster dose is to protect yourself against COVID-19?" 1. This question offers a scale of values "1"-"5", with the answer range being not at all important, not too important, somewhat important, very important, and extremely important. (It will be identified as "Booster".)

The responses to these survey questions were compared against their nasal swab sample PCR results.

DATA ORGANIZATION AND STATISTICAL ANALYSIS

A descriptive analysis was performed to identify how the different components of the participant data set could be broken down based on sex, age group, education, and income level. Furthermore, these participants were identified by

whether they had received their first vaccination, as well as if they had been infected at least once with COVID-19 by the end of the study period in December 2022. Participants were asked to report their vaccination status by submitting their vaccination cards and were designated as being vaccinated if they had become vaccinated before (greater than 3 months prior to enrollment) or during their participation in the study.

To identify a change in perception, the original set of data was composed of the numerical answers to the three survey questions, corresponding survey dates when the participant took the survey, and the dates of any identified positive infections. All data from participants who did not receive a PCR-positive result were categorized as "negative"; participants who received a PCR-positive result were categorized as "positive". To determine a change in perceptions over time, a difference-in-difference was calculated. For both groups, the initial difference calculated was the final survey results (Survey 7) to the initial survey responses (Survey 2). These results were then compared to each other by running a difference-in-difference study, with a p-value of 0.05 being used to determine statistically significant results.

RESULTS

The data for 693 RECOVER participants were identified and gathered. Participant characteristics are summarized in Tables 1 and 2, with Table 1 comparing participants who had received a vaccination or were unvaccinated and Table 2 comparing infected or non-infected participants. Participants included a 1:3 ratio of males to females, 88.89% of participants were between the ages of 18-49, 69.40% had obtained a college degree or an additional graduate or professional degree, and 57.00% were within the income brackets of $25,000-124,999.

When a differences-in-differences analysis was performed,

it was determined that the results of CovidChance were statistically significant with a difference-in-difference estimate of -0.325 and a p-value of 0.0478. This indicates that after a participant tested positive for COVID-19, their perception of a future infection did not increase to the same degree as did those that did not have a COVID-19 infection. The resulting model and statistical analysis for CovidChance are in Graph 1 & Table 3.

The result of CovidWord was trending towards statistical significance with a value of -0.227 with a p-value of 0.0744; the model and statistical analysis are in Graph 2 and Table 4. This conclusion indicates that after a participant tested positive for COVID-19, they were potentially less concerned or worried about prospective positive COVID-19 infection in the future than those who did not have a COVID-19 infection. The similarity in numerical value indicates a similar sentiment to the results of CovidChance, but further investigations must be conducted to identify a statistically significant comparison.

The result analysis of Booster was a value of -0.161, with a p-value of 0.530. Because the result of the Booster analysis was not statistically significant, it could not be determined if there was a clear association between a willingness to become vaccinated and a participant's testing status. To better identify if there is such an association, additional analyses with a more diverse and large population should be conducted. The model and statistical analysis for Booster are found in Graph 3 and Table 5.

Table 1: A demographic comparison of participants who had received a vaccination or were unvaccinated.

Sex		Received Vaccination	Unvaccinated		Income Level		Recieved Vaccination	Unvaccinated
Sex	Male	35.70% (136)	40.38% (126)		Income Level	$0-$24,999	3.94% (15)	1.92% (6)
	Female	64.30% (245)	59.62% (186)			$25,000-$49,000	15.22% (58)	15.06% (47)
Age Group	18-29	33.07% (126)	23.40% (73)			$50,000-$74,999	17.32% (66)	11.54% (36)
	30-39	46.72% (178)	26.60% (83)			$75,000-$99,999	14.70% (56)	13.46% (42)
	40-49	13.91% (53)	33.01% (103)			$100,000-$124,999	12.60% (48)	13.46% (42)
	50-59	2.36% (9)	14.74% (46)			$125,000-$149,999	4.99% (19)	9.94% (31)
	60-69	3.41% (13)	1.92% (6)			$150,000-$174,999	6.56% (25)	5.13% (16)
	70-79	0.52% (2)	0.32% (1)			$175,000-199,999	1.84% (7)	5.45% (17)
Education	No Diploma	0.26% (1)	0.32% (1)			$200,000+	8.66% (33)	10.90% (34)
	High School/GED	4.46% (17)	4.17% (13)			No Response	14.17% (54)	13.14% (41)
	Some College	20.73% (79)	21.47% (67)					
	College Degree	41.47% (158)	41.67% (130)					
	Graduate/Professional	28.61% (109)	26.92% (84)			Overall Sample	381	312
	No Response	4.46% (17)	5.45% (17)					

Table 2: A demographic comparison of infected or non-infected participants. Note that the phrase "Infected" represents individuals that have been infected at least once during the examination period

Sex		Infected	Noninfected		Income Level		Infected	Noninfected
Sex	Male	38.11% (165)	37.31% (97)		Income Level	$0-$24,999	3.93% (17)	1.54% (4)
	Female	61.89% (268)	62.69% (163)			$25,000-$49,000	16.40% (71)	13.08% (34)
Age Group	18-29	23.56% (102)	37.31% (97)			$50,000-$74,999	16.86% (73)	11.15% (29)
	30-39	40.42% (175)	33.08% (86)			$75,000-$99,999	15.01% (65)	12.69% (33)
	40-49	24.02% (104)	20.00% (52)			$100,000-$124,999	13.16% (57)	12.69% (33)
	50-59	8.55% (37)	6.92% (18)			$125,000-$149,999	8.31% (36)	5.38% (14)
	60-69	3.23% (14)	1.92% (5)			$150,000-$174,999	6.00% (26)	5.77% (15)
	70-79	0.23% (1)	0.77% (2)			$175,000-199,999	4.85% (21)	1.15% (3)
Education	No Diploma	0.46% (2)	0.00%, (0)			$200,000+	11.55% (50)	6.54% (17)
	High School/GED	3.70% (16)	5.38% (14)			No Response	3.93% (17)	30.00% (78)
	Some College	23.09% (100)	17.69% (46)					
	College Degree	42.49% (184)	40.00% (104)					
	Graduate/Professional	30.02% (130)	24.23% (63)			Overall Sample	433	260
	No Response	0.23% (1)	12.69% (33)					

Graph 1: The difference-in-difference model for CovidChance
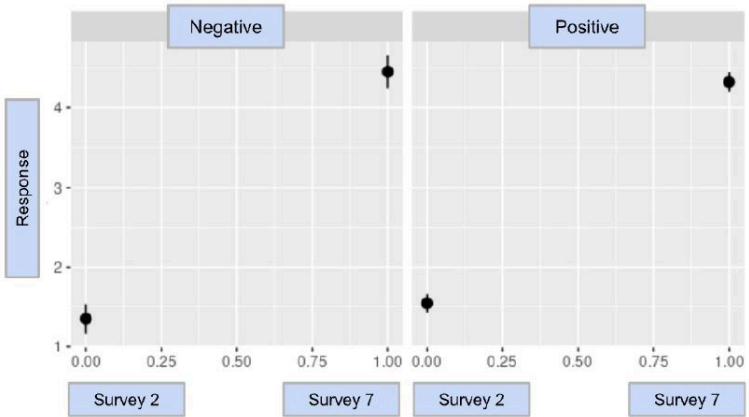
Graph 2: The difference-in-difference model for CovidWord
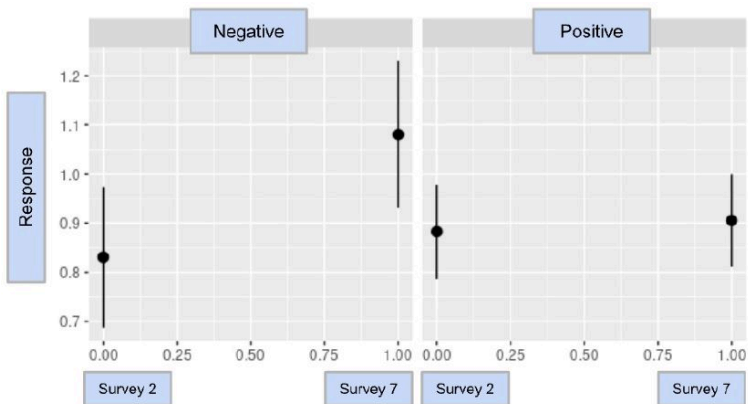
Graph 3: The difference-in-difference model for Booster
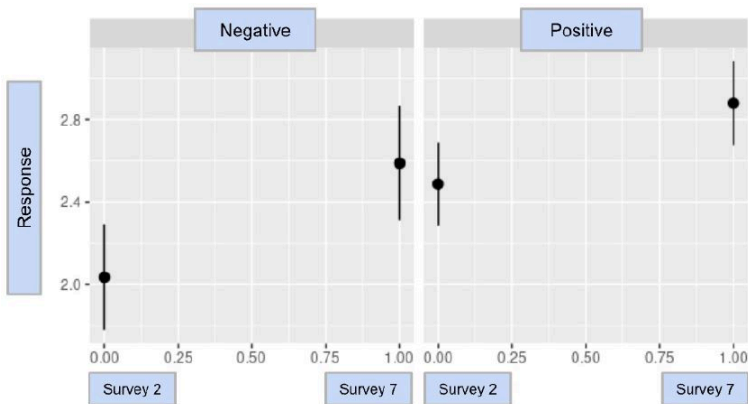
Table 3: The difference-in-difference analysis for CovidChance; the p-value is 0.0478

	Survey 2	Survey 7	Difference
Negative	1.35	1.545	0.195
Positive	4.45	2.645	-0.13
Difference	3.1	2.775	-0.325

Table   4: The difference-in-difference analysis for
CovidWord; the p-value is 0.0744

	Survey 2	Survey 7	Difference
Negative	0.831	0.8827	0.0517
Positive	1.081	-0.1523	-0.175
Difference	0.25	0.023	-0.227

DISCUSSION

This study examined the relationship between the
notification of a positive COVID-19 infection and participant
perception of COVID-risk and vaccine hesitancy within a study
population of the RECOVER study program. It was determined
that during the time period of March 31st, 2021 to December
16th, 2022, participants who tested positive did not have as
sharp of an increase in their estimated probability of the
potential of testing positive for a second time. Despite the
statistical significance, the resulting value of -0.325 indicates
that the difference in responses between the two groups may
not have much clinical significance.

Given the complexity of vaccine hesitancy noted in the
literature, one's recent COVID-19 status is only one aspect
of any person's perception of the future risk and need for
immunization. Participant awareness of information spread by
the media at the time could cause a change in perception. At
the start of the examination period, a weekly epidemiological
update for the week of March 30th, 2021 placed new cases of
COVID-19 global infections at nearly 3.8 million (World, 2021);
for the week of December 5th, 2022, there were over 3.3 million
new cases of infection (World, 2022). This information would
have been easily and digitally accessible to any interested

individual, including participants in the RECOVER study who were asked to take three-month surveys at this time. With the demographics of this specific study primarily being in the age range of 18-49, and in conjunction with the previously referenced "Covid Infodemic", it is likely that our participants were aware of the declining rates of infection during this time period.

Furthermore, in terms of the examination time frame, participant perceptions are likely to have been greatly swayed in a significant way due to the introduction of additional COVID-19 variants, such as the Omicron variant. The CDC identified the Omicron variant on November 26th, 2021, which was determined to be the most common variant, replacing the Delta variant. Moreover, the Omicron variant was estimated to be "more predisposed to causing transmission before symptom onset than Delta" (Zeng, 2023). Notably, the identification of the Omicron variant occurred near the halfway point of this study's study time frame; despite new variants, participants who had a prior infection appeared to feel confident in the native immunity acquired from the infection and felt they had a reduction in risk for a future occurrence. It may be that the presence of a widely publicized new variant decreased the perceived effectiveness of prior native immunity leading to a smaller effect size, however, survey questions were limited in their scope to evaluate such questions.

Given that infections from different pathogens will trigger native immunity of varying levels of effectiveness, it is important to recognize cases where native immunity is inherently ineffective, particularly when there is an available vaccination. In such cases, results seen in this study, as well as in the literature (Do, 2022), indicate that clear messaging needs to be provided to the general population to help them

understand the true level of protection of both native immunity as well as that which can be provided by vaccinations.

As noted above, a comparison of the survey results regarding concern or anxiety regarding a future infection did not meet statistical significance, though the p-value was trending towards significance for a lower value in the infected group. In comparison to the difference-in-difference with the other questions, the slope in this analysis visually was different; however, the large confidence intervals likely led to the p-value being non-significant. Given the similarities between the two survey questions (CovidChance and CovidWord), it is not surprising that both results are trending in a similar direction.

It is important to distinguish the fact that there is a wide range of limitations that can cause discrepancies in this particular set of data results. Beyond the direct analysis of blood serum and nasal swab samples, the RECOVER study is dependent on our participants to self-report infections, vaccinations, symptoms, personal information, and much more. Thus, there is an inherent chance that there could be inaccurate reports resulting from participant perception, misunderstanding, reluctance, and/or refusal to share personal information. For example, when participants are asked if they have any potential symptoms of an infection, there are subjective differences in any one individual's perception as they report their results. In addition, at the most basic level, a participant's refusal to complete a survey, blood draw, or nasal swab sample will create data inaccuracies. At the height of the pandemic, it was recommended by the Occupational Safety and Health Administration (OSHA) that employers should "instruct any workers who are infected, unvaccinated workers.. and all workers with COVID-19 symptoms to stay home from work" (Occupational, 2021). Hesitancy to report symptoms to the

RECOVER Study can thus be potentially linked to a fear of missing work time and subsequent income loss. Finally, while this examination does not use the results of blood serum samples, hesitancy or fear of blood collection can also translate to an unwillingness to complete surveys and weekly nasal swab samples that participants are asked to complete at their own convenience.

In terms of the nasal swab samples, there can be a failure to collect these samples due to the participant or their assigned courier missing the weekly swab collection. A missed swab sample will result in a missed PCR test, which if it was a positive sample, results additionally in the loss of a completed active surveillance form that is sent out to any participant who has either tested positive for COVID-19 or has reported potential symptoms of infection. Furthermore, because a PCR-based test will detect viral genetic material, which can "stay in (the) body for up to 90 days after (testing) positive" (Centers, 2022), the continued weekly testing of nasal swab samples paired with the participant being notified in the same manner will potentially cause a skew in perception even if the participant no longer considered infectious.

The demographics of the study population are not well-balanced in the representation of a normal population, which presents another limitation. An inspection of general RECOVER study participant demographics indicates that the majority of the study population identified themselves to be white and female, with most individuals possessing a college degree or beyond. As a result, this study may not be generalizable to those beyond the demographics represented in this study.

Finally, conducting a differences-in-difference analysis requires that there is a parallel trends assumption for our

model. This requires the assumption that if all RECOVER participants had never tested positive for COVID-19, their perceptions concerning vaccine hesitancy would be constant. While there is inherent variability, as noted above there are parallel trends in the data, therefore, the differences-in-differences analysis appears to be an appropriate tool for an examination of the changes in outcome over time within our population.

The strengths of the study include the large population that was involved in the RECOVER, of which the vast majority were engaged with the surveys throughout the duration of the study. While the above limitations of the PCR testing are discussed above, the consistent PCR testing of the participant nasal swab samples provides relatively timely feedback to participants regarding their COVID-19 status, as opposed to other designs which may rely more upon clinical signs and symptoms. Finally, the prospective nature of our study eliminates a number of the biases that may be seen in other, retrospective, or cross-sectional studies, and provides an accurate picture of the change in the participants' perceptions of these survey questions.

CONCLUSION

This study identified changes in RECOVER participant perception of the importance of vaccine uptake, as well as the risk and severity of future COVID-19 infection. Based upon results from our study, prior infection appears to influence one's perspective with regards to the likelihood of future infection; the literature suggests that this may be true for a time, but as we have seen over time, new variants and waning immunity may lead to an underestimation of future risk. By contributing to the growing body of literature related to this particular subject, there will be more confidence in improving

future medical practices towards vaccination and related education to the general public, with a greater understanding of how differently those with native infections may perceive the need for vaccination compared to those who have yet to be infected. Perception should be studied in the future in regard to other factors such as individual health and pre-existing medical conditions to identify if other aspects contribute to vaccine hesitancy, as well as to help direct public health efforts toward improving vaccination rates.

REFERENCES

Cavanaugh, A. M., Spicer, K. B., Thoroughman, D., Glick, C., & Winter, K. (2021, August 13). *Reduced Risk of Reinfection with SARS-CoV-2 After COVID-19 Vaccination – Kentucky, May-June 2021.* Morbidity and Mortality Weekly Report. https://pubmed.ncbi.nlm.nih.gov/34383732/

Centers for Disease Control and Prevention. (2022, September 28). *COVID-19 Testing: What You Need to Know.* Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/testing.html#:~:te xt=NAATs%2C%20such%20as%20PCR%2Dbased,days%20after% 20you%20tes t%20positive.

Centers for Disease Control and Prevention. (2023a, March 20). *SARS-CoV-2 Variant Classifications and Definitions.* Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html

Centers for Disease Control and Prevention. (2023b, April 12). *Covid-19 Vaccinations in the United States.* Centers for Disease Control and Prevention. https://covid. cdc.gov/covid-data-tracker/#vaccinations_vacc-total-admin-count-total

Do, D. P. & Frank, R. (2022). *Prior COVID-19 infection: an underappreciated factor in vaccine hesitancy in the USA.* Oxford

University Press Public Health Emergency Collection. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8807188/

Dubé, E & MacDonald, N. E. (2022). *COVID-19 vaccine hesitancy.* Nature Reviews Nephrology. 18, 409-410. https://doi.org/10.1038/s41581-022-00571-2

Fieselmann, J., Annac, K., Erdsiek, F., Yilmaz-Aslan, Y., & Brzoska, P. (2022). What are the reasons for refusing a COVID-19 vaccine? A qualitative analysis of social media in Germany. *BMC Public Health.* 22(846). https://doi.org/10.1186/s12889 -022-13265-y

Jasarevic, T. (2015, August 18). *Vaccine hesitancy: A growing challenge for immunization programmes.* World Health Organization. https://www.who.int/ news/item/ 18-08-2015-vaccine-hesitancy-a-growing-challenge-for-immunization-programmes

Morens, D. M., Folkers, G. K., & Fauci, A. S. (2022). *The Concept of Classical Herd Immunity May Not Apply to COVID-19.* The Journal of Infectious Diseases. 226(2). https://doi.org/10.1093/infdis/jiac109

Occupational Safety and Health Administration. (2021, June 10). *Protecting Workers: Guidance on Mitigating and Preventing the Spread of COVID-19 in the Workplace.* Occupational Safety and Health Administration. https://www.osha.gov/coronavirus/safework

Pooley, N., Karim, S. S., Combadière, B., Ooi, E., Harris, R. C., Seblain, C., Kisomi, M., & Shaikh, N. (2023). *Durability of Vaccine-Induced and Natural Immunity Against COVID-19: A Narrative Review.* Infectious Diseases and Therapy. 12(2). https:// doi.org/10.1007/s40121-022-00753-2

Sekizawa, Y., Hashimoto, S., Denda, K., Ochi, S., & So, M. (2022). *Association between COVID-19 vaccine hesitancy and generalized trust, depression, generalized anxiety, and fear of*

COVID-19. BMC Public Health. 22(126). https://doi.org /10.1186/s12889-021-12479-w

University of Utah Health. (2023). *Who Can Participate.* University of Utah Health. https://medicine.utah.edu/dfpm/ occupational-environmental-health/research/recov er/who-can-participate Wilder-Smith, A. (2021). *COVID-19 in comparison with other emerging viral diseases: risk of geographic spread via travel.* Tropical Diseases, Travel Medicine and Vaccines. 7(3). https://doi.org/10.1186/s40794-020-00129-9

Wiysonge, C. S., Ndwandwe, D., Ryan, J., Jaca, A., Batouré, O., Anya, B, & Cooper, S. (2022). *Vaccine hesitancy in the era of COVID-19: could lessons from the past help in divining the future?* Human Vaccines & Immunotherapeutics. 18(1). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8920215/

World Health Organization. (2020, December 31). *Coronavirus disease (COVID-19): Herd immunity, lockdowns and COVID-19.* World Health Organization. https://www.who.int/ news-room/questions-and-answers/item/herd-immunity-lock downs-and-covid-19#:~:text=The%20percentage%20of%20people%20who,a mon g%20those%20who%20are%20vaccinated.

World Health Organization. (2021, March 30). *Weekly epidemiological update on COVID-19 – 30 March 2021.* World Health Organization. https://www.who.int/publications/m/ item/weekly-epidemiological-update-on-covi d-19—31-march-2021

World Health Organization. (2022, December 14). *Weekly epidemiological update on COVID-19 – 14 December 2022.* World Health Organization. https://www.who.int/publications/m/ item/weekly-epidemiological-update-on-covi d-19—14-december-2022#:~:text=Download%20(1.2%20MB)- ,Overview,over% 209700%20new%20fatalities%20reported.

Zeng, K., Santhya, S., Soong, A., Malhotra, N., Pushparajah, D., Thoon, K. C., Yeo, B., Ho, Z. J., & Cheng, M. C. (2023). *Serial Intervals and Incubation Periods of SARS-CoV-2 Omicron and Delta Variants, Singapore.* Emerging Infectious Diseases. 29(4), 814-817. https://doi.org/10.3201/eid2904.220854.

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67. Research Reflection by Katie Luong

Katie Luong

Faculty Mentor: Andrew Phillips (Family & Preventative Medicine, University of Utah)

I have been fortunate enough to have worked in various research laboratories; the circumstances of the COVID-19 pandemic resulted in my current placement within the RECOVER Study, where I have been a researcher and phlebotomist for over a year and a half. Because this research program is not your typical wet lab, I gained a new perspective on the pandemic and alternate data-gathering methods. In all, I hope to use the knowledge I have acquired within this study to become a better healthcare worker and to better prepare for my entrance into medical school and beyond.

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68.   **Barriers with Recruitment and Retention of Diverse Populations in Psychological Research**

Melika Moeinvaziri and
Scott Langenecker
(Psychiatry)

Faculty Mentor: Scott Langenecker (Psychiatry, University of Utah)

Introduction/Background

Psychological Research has struggled to recruit and retain individuals of more diverse populations. While diverse

populations contribute valuable information to research, many individuals of these backgrounds do not participate in research. Many of these research labs' participants are white and wealthy, which does not match up with the average population's demographics. Participants in research should reflect the diversity of our culture and conditions, taking into account race, ethnicity, gender, age, etc. The lack of diversity among research participants has serious ethical and research consequences (Palmer & Burchard (2022)). Research demographics should reflect the diverse population that we have in the United States and all over the world. The purpose of this study is to examine the barriers to the recruitment and retention of a diverse population in psychological research. This study will examine how these barriers affect psychological research and how we can improve access and retention of more diverse populations.

Demographics of the Lab

Native Hawaiian or Other Pacific Islanders 1%
Hispanic/Latinx 9%
Indigenous Peoples 1%
Asian 4%
Other 1%
White 85%
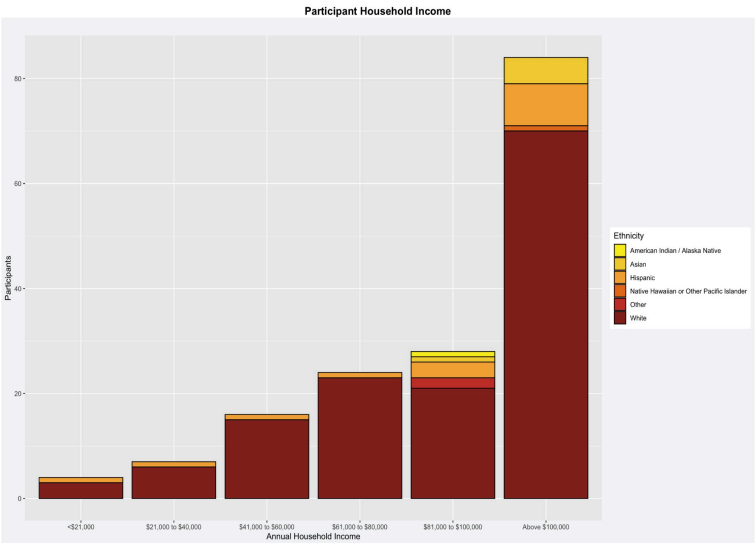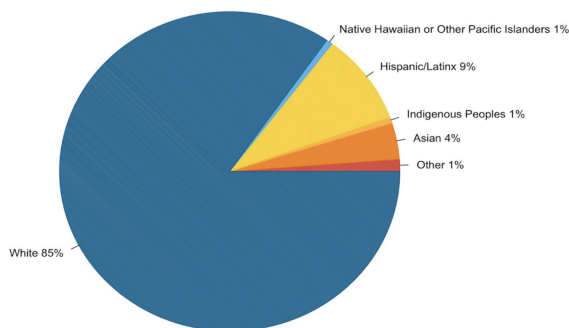
Lab Experiences/Anecdotes

Recruitment

- What have you noticed works best with keeping people involved in studies?

- Tenacity, patience, and flexibility with teens. -What is most important to consider when recruiting for a study?

- To not have recruitment bias, such as only recruiting from one school district or only targeting certain populations that are not meant to be targeted (recruiting only white people when we mean to recruit a diverse representative sample) -How do you plan on recruiting more people of color into your studies?

- We are looking to continue to partner with community agencies. We will look to advertise directly to youth from minority communities. We

will create social media and web materials to
promote our drive toward inclusivity and diversity.
We will work with volunteers and youth from POC
communities to brainstorm strategies.

Retention:

- What have you noticed works best with keeping
  people involved in studies?

- I think efficiency is appreciated, if we lose contact
  with them or aren't flexible with scheduling they
  will often not want to keep in touch as we are
  showing them they are unappreciated or not a
  priority.

- With POC, Have you noticed some things work
  better than others when trying to keep them
  involved?

- I think many of our efforts are more cookie-cutter
  and not necessarily targeted to keep certain groups
  in the study. So it seems to me that flexibility and
  extra communication are things that are more
  naturally used in families who seem to be struggling
  interpersonally or financially, and these families are
  unfortunately disproportionately POC.

Ways to Improve

To better retention with those lower SES, we can offer
hardship payments and other types of compensation. Face-to-
face events can also help families be able to see and interact
with labs and understand what the labs are doing.

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69. **Identification of 42 Potential Colorectal Oncogenes via a RNAi Screen in Drosophila Melanogaster**

Carter Niedert; Bruce Edgar (Oncological Sciences); and Peng Zhang (Oncological Sciences)

Faculty Mentor: Bruce Edgar (Oncological Sciences, University of Utah)

Abstract

Colorectal cancer is one of the most common and deadly

diseases in the world. Colorectal cancer often arises from a mutated or damaged gene that leads to uncontrolled cell proliferation in the intestines. Controlling this cell proliferation by targeting the mutated or damaged genes is a potential form of cancer treatment with advantages over current treatment options. The goal of this project was to identify genes involved in damage-mediated cell proliferation which could serve as potential targets for novel cancer treatments. Using a technique known as a RNAi knockdown screen with Drosophila Melanogaster, this project found that 42 of 83 tested genes were shown to play a role in damage-mediated cell proliferation. Specifically, 14 genes were identified as promoters of cell proliferation and 28 as inhibitors. These results lay the foundation for future research that could develop methods to target these genes in colorectal cancer cells to treat patients. Such treatments could significantly improve the prognosis for colorectal cancer patients and reduce the burden of this disease on global public health.

Index Terms— Cell signaling, Gastroenterology, Genetics, Tumors, RNAi.

I. INTRODUCTION

Colorectal cancer is the third most common cancer in the United States and the second leading cause of cancer related deaths [1]. Cancer deaths occur due to the disruption of normal physiological processes caused by rapid and uncontrolled cell proliferation. Cell proliferation in cells is often regulated through cellular damage sensing pathways [2]. In the intestines, damage sensing pathways are activated by specific genes. A better understanding of the genes that are involved in intestinal damage sensing pathways could lead to possible colorectal cancer treatments that dampen these pathways and control cell proliferation.

Cell proliferation occurs rapidly in the gut due to constant exposure to stressors from the external environment [3]. Cells in the gut can sense damage caused by these stressors and trigger renewal of gut epithelial tissues via intestinal stem cell (ISC) proliferation [4]. Damaged intestinal cells can trigger ISC proliferation by releasing ligands that activate Jak-Stat signaling proliferation pathways [5]. Jak-Stat pathways mediate cytokine signaling and cytokines are commonly believed to be the main factor for activating ISC proliferation [6], [7]. Overexpression of certain Jak-Stat signaling factors has been linked to cancer development in the intestines [7]. While Jak-Stat presents an interesting target for potential cancer therapies, there are a multitude of other pathways are also involved the regulation of ISC proliferation that could be potentially targeted. For example, Wnt family signaling pathways use β-catenin as their main effector to increase or decrease ISC proliferation by activating factors such as c-Jun N-terminal kinase (JNK) or interacting with tumor suppressor genes such as APC [8], [9]. Mutations in APC and overexpression of Jak-Stat or JNK factors can lead to cancer formation and rapid cell proliferation. Due to the complexity of cellular signaling pathways, there are a staggering number of potential oncogenes that have been identified but are not yet fully understood in the context of intestinal damage-sensing. A few examples of these oncogenes include RTN1 (a type of reticulon encoding gene linked to endoplasmic reticulum function), PIEZO1 (produces a protein that links mechanical forces to biological signals), and JUND (can protect cells from p53-dependent senescence and apoptosis)[10]. These few potential oncogenes represent a much larger number of known and unknown genes that cancer cells could use to rapidly grow and evade cell death.

There are many potential cancer genes involved in damage sensing pathways and ISC proliferation that remain unidentified or poorly understood. Such genes are likely responsible for the regulation of ligand release that initially activates pathways such as Jak-Stat or Wnt [7], [9], [11]. The lack of knowledge about the function of and factors released by these potential oncogenes prevents targeted gene treatments and obscures potentially novel treatment options. An example of this is the p38 signaling pathway, which researchers have only recently discovered [12]. Researchers were able to find that damage triggers a certain signaling pathway that leads to eventual p38 activation and causes increased cell division. However, researchers were unable to understand exactly how p38 senses damage or stress and what factors it releases to promote regeneration. Understanding what factors allow p38 to sense stress could lead to physicians being able to artificially regulate the p38 pathway in cancerous cells to diminish cell growth and spread. Understanding more about other genes such as p38 could lead to discoveries about other unknown mechanisms beyond

The aim of this project is to identify which genes in the gut are responsible for sensing damage and activating cell proliferation. Disabling a gene suspected in cell proliferation pathways and quantifying the effect of its inhibition on cell proliferation is a proven method for studying gene effects. This will be done in this project by performing an RNAi gene knockdown screen using Drosophila melanogaster (i.e., fruit flies). This screen will be performed on 82 genes that have been identified through literature as being potentially involved in intestinal damage sensing pathways, such as inflammation or chemical stress pathways [14], [15]. The results of this screen could lead to a deeper understanding of damage- University

of Utah UNDERGRADUATE RESEARCH JOURNAL sensing signaling pathways and to novel cancer treatments that can target specific genes. Targeted treatment of these identified genes could prevent colorectal cancer cells from rapidly proliferating and prevent them from causing the death of the patient.

II. BACKGROUND

Cancer refers to the rapid and uncontrolled growth of cells within the human body. This rapid cell growth usually arises from a mutation in a gene involved in regulating the cell cycle or programmed cell death (also known as apoptosis) [16]. Cancer cells that evade apoptosis are able to pass membranes and barriers healthy cells would not, continue undergoing cell division, and disrupt normal physiological processes [15]. This disruption can lead to death by organ failure, visceral infections or septicemia, infarction, and internal hemorrhaging [17].

Just like in healthy cells elsewhere in the body, cell division in ISCs consists of two heavily regulated stages called interphase and mitotic phase. The initiation of cell division can be triggered by several factors such as nearby cell death or damage [18]. During interphase the cell grows, replicates its DNA, and prepares to divide. The mitotic phase consists nuclear division and the physical separation of the cell into two daughter cells [19]. These daughter cells can be more ISC cells, absorptive enterocytes, goblet cells, enteroendocrine cells, or Paneth cells, each of which perform a different and necessary function in the gut [20]. In intestinal stem cells and most cells in the body, regulatory proteins known as cyclin-dependent kinases (CDK) activate at various checkpoints throughout the cycle and allow the cell to continue to subsequent stages of cell division [21]. Genes that produce or regulate the production of CDK inhibitors (CKI), such as p15, p27, p53, and p57, are able

to inhibit CDKs which halts the progression of the cell cycle and prevents cell proliferation [19], [22]. CDK inhibition can often be triggered by sensed DNA damage [22]. Mutations in the genes that regulate CDK and CKI production often leads to the development of cancer as cells with DNA damage and mutations will proceed with cell division when inhibition by CKIs would otherwise prevent them from doing so [23]. Such mutations can be caused by exposure to pesticides, air pollution, metal fluids, and other chemicals [24].

Intestinal/colorectal cancer is the third most common type of cancer, likely due to the high turnover rate of the intestines and constant exposure to ingested chemicals providing more opportunity for cell mutations to occur [25]. In fact, the epithelial lining of the intestine replaces all of its cells every few days [4]. Some studies have also shown that inflammation due to illnesses such as ulcerative colitis and Crohn's disease is another potential risk factor for developing colorectal cancer [26], [27]. Initial studies that aimed to understand cell damage and renewal in the intestines investigated the cells responsible for intestinal cell renewal, stem cells [28], [29]. Discoveries was made in 2009 and 2010 showed that specific cells called crypt stem cells were the cells-of-origin of intestinal cancer, meaning that a few mutated crypt stem cells that are able to self-renew and exhibit multipotency are responsible for the formation of large malignancies in the intestines [30], [31]. The same studies also showed the deletion of the tumor suppressor gene APC, which is also involved in Wnt-mediated proliferation pathways, led to generation of cancerous stem cells [9], [31]. Such findings led to an increased interest in understanding the pathways and genes that regulate stem cell proliferation in the intestines. A better understanding of these pathways that cancerous stem cells use to self-renew and proliferate could

allow researchers and clinicians to develop treatments that target such pathways and eliminate the tumor's self-renewing ability [32].

Chemotherapy is currently one of the most common treatments for intestinal (and many other types) of cancer [33]. Chemotherapy for colorectal cancer treatment became feasible in 1957 with the development of a compound called 5-fluorouracil (5-FU) that inhibited tumor cell division [34]. 5-FU functioned by blocking biosynthesis of essential nucleic acids in cancer cells, removing their ability to grow and divide [35]. Throughout the 1970's and 80's, clinical trials were performed to study the effectiveness of 5-FU treatment [36], [37]. These trials identified improved forms of treatment that generally consisted of administering 5-FU with varying amounts of a different compound (such as leucovorin). Also during the 1980's, other treatments that inhibited epidermal growth factor were discovered and shown to be effective at limiting cancerous cell growth [38], [39]. Different compounds and chemotherapeutic treatments continued to be discovered and refined throughout the 20th and 21st century, but chemotherapy side effects such as extreme nausea, vomiting, diarrhea, long-lasting peripheral neuropathy, and severe toxicity to healthy cells make chemotherapeutic treatment a far from perfect solution [40]–[43]. This motivates the need to find an effective cancer treatment that is less harmful to the patient.

Personalized cancer treatment has become an increasingly more feasible and potentially efficacious method of treatment in recent years [44], [45]. Personalized treatment refers to determining the most effective pharmacological approach for a patient based on their own molecular characteristics [46]. Though such treatment requires the expensive process of whole-genome sequencing for each patient, its ability to

identify target genes for treatment and increasing cost-effectiveness make personalized treatment more feasible each day [47], [48]. However, the complexity of cellular pathways and the many unknown genes involved in regulation of cell growth and division limit researchers' ability to develop individualized treatments. A better understanding of cellular pathways and genetic factors would increase the feasibility of personalized cancer treatments [49], [50].

Drosophila melanogaster, also known as fruit fly, has been historically used as a powerful model to better understand physiological processes and genetic disease in humans [51]. Approximately three-fourths of all genes responsible for disease in humans have homologs in *Drosophila* [52]. *D. melanogaster* can be used as a model for the study of intestinal stem cell physiology due to the similarities of the intestinal development signaling pathways between *D. melanogaster* and mammals [53]. Beyond its genetic similarity to mammals, *D. melanogaster* is also a desirable model due to its quick life cycle and simple genetics. At 25 ℃, it takes just 9-10 days for *D. melanogaster* to develop from a fertilized egg to an adult and their genome only consists of four pairs of chromosomes [54]. These facts make it relatively easy and simple to breed flies with desired genes and to perform timely experiments [55].

RNAi interference is a method to perform gene knockdown which allows for the examination of any effects that a given gene may have on cell proliferation [56]. Active RNAi will cause the production of siRNA that will bind to the mRNA of a given gene. The immune system will recognize and destroy the siRNA/mRNA complex, preventing the translation of the corresponding protein and effectively silencing the targeted gene [57]. Since knocking down the function of a gene can be lethal, this method relies on the interaction between Gal80,

Gal4, and UAS. Gal4 has little to no effect by itself as its main function is to bind to UAS [58], [59]. Once Gal4 has bound to UAS, the gene bound to UAS will be activated. Pairing UAS with RNAi results in the siRNA production and gene knockdown. Therefore, by crossing fly lines with Gal4 and UAS that is bound to a specific gene, the offspring will have that specific gene silenced. Gal80 is used to inhibit Gal4 at temperatures under 29 ℃ where the

flies can be crossed and maintained. Once the effects of gene knockdown are ready to be observed, placing the flies into an incubator above 29 ℃ will inhibit Gal80 and allow Gal4 to drive the previously described gene knockdown by RNAi interference [60].

RNAi screens are a popular technique for observing the effects of gene knockdown. Initial excitement for this technique was tempered by the prominence of false positives caused by off-target effects [61], [62]. However, simple solutions such as re-screening or comparing results of multiple screens through meta-analyses can verify the results of a given screen [62]. This means RNAi screens are still a convenient way to perform high-throughput experiments to identify genes of interest for a given signaling pathway or system response.

III. METHODS

A. Materials

The flies used in this project were lines of *Drosophila Melanogaster* with certain UAS-RNAi complexes already inserted in their genetic code. These flies were obtained from the Vienna Drosophila Resource Center in Vienna, Austria. One "driver" line of flies with a Myosin1A-Gal4 UAS-GFP tub-Gal80 complex was used in crosses with the RNAi lines to produce offspring with a complete system for gene silencing. This driver line was obtained from the Bloomington Drosophila

Stock Center in Bloomington, Indiana. Myosin IA (MyoIA) was selected as a driver since it is mainly expressed in *Drosophila* enterocytes (mature intestinal cells) and so gene knockdown would be localized to the intestines [11]. Since UAS-GFP was included in the driver line as well, selected offspring would have both GFP fluorescence and RNAi production in the gut when shifted to 29 ℃ [63]. This allowed GFP fluorescence to serve as an indicator that the desired RNAi production and gene knockdown mechanisms were successfully incorporated into the offspring flies.

Both driver and RNAi lines were obtained with a "balancing" gene called CyO [64]. This meant that the fly lines could either pass on the driver system, UAS-RNAi complex, or the CyO gene to their offspring. Flies with two copies of the CyO gene were nonviable and quickly died. The inclusion of this balancer thus serves two purposes. First, it preserves the desired driver or RNAi system as flies breed during storage as offspring without the desired system are unable to survive or reproduce. Second, the CyO gene produces a phenotype of fly with "curly" wings. This allows visual inspection of the offspring from crosses between the driver and RNAi lines to determine if the gene knockdown system is in place (the fly has straight wings) or if one component of the knockdown system is missing (the fly has curly wings and is missing either the driver or RNAi complex).

A bacterial culture of *Pseudomonas entomophilia (Pe)* was used in this project to stress the intestinal cells of the flies. This culture was prepared by performing a 1:1000 dilution of *Pe* in Luria-Bertani (LB) broth and placing the culture in a rotating incubator at 30 ℃ and 180 rpm for 26-30 hours. The resulting culture was centrifuged at 4 ℃ at 3000rpm for 15 minutes, supernatant was discarded, and the precipitate was collected.

The precipitate was mixed with a 10% sucrose in water solution to produce 400μL of "bacterial solution" for each cross that would be infected.

To make the LB broth used in the *Pe* culture, 950mL of water, 10g of tryptone, 10g of NaCl, and 5g of yeast extract were combined in a large glass container. The mixture was shaken and adjusted to a pH of 7.0 using sodium hydroxide. The broth was then sterilized in an autoclave for 25 minutes at 120 ℃.

Cell staining and visualization was achieved using DAPI, Alexa Fluor 488 Goat anti-Chicken antibodies, and Phospho-Histone H3 (pH3) Polyclonal antibodies obtained from Thermo Fisher Scientific, Salt Lake City, Utah. DAPI stained all present DNA for visualization of the nuclei within the gut. Alexa Fluor 488 stained enterocytes with the gene knockdown system in place and served to verify that fly crosses had been successful. pH3 allowed for visualization of cells undergoing division.

Several other different chemicals and compounds were used to prepare the fly guts for visualization. Extracted fly guts were suspended in a 1x phosphate buffered saline solution (PBS). PBS kept intestinal cells from rupturing or shriveling due to osmosis before they could be fixed. Fixation was achieved using a 4% paraformaldehyde (PFA) solution. PFA causes covalent cross-links between molecules which prevents cell decay or putrefaction. Triton X-100 diluted to 0.2% in 1x PBS (final solution called 0.2% PBST) was used to wash guts in between fixation and immunostaining steps. Triton X-100 is a nonionic surfactant that, when added to tissue that has been fixed in PFA, permeabilizes tissues prior to blocking and immunostaining. This improves the fidelity of following immunostaining. Lastly, a product called VECTASHIELD Antifade Mounting Media was placed on the final slides where

guts were mounted to preserve the fluorescence of the antibodies.

Fly stocks and crosses were kept and maintained in plastic vials with about 10mL of a proprietary cornmeal-based food. Flies were kept in an 18℃ incubator when not undergoing experiments or maintenance.

B. Fly Crosses, Infection, and Dissection

For each batch performed in the screen, 10-15 virgin females of the driver line were collected and crossed with flies from one of the RNAi lines. One cross between the driver line and wildtype flies with no gene knockdown system was also done to serve as a control for each particular batch. Adult flies were removed from the vials where the crosses occurred after one week had elapsed to ensure that only pupae generated from the cross remained in each vial. Vials were kept in an 18℃ incubator for a total of about 20 days which is when the first generation of fly progeny would eclose (emerge as an adult from the pupa). Offspring with the gene knockdown system (which were identified by the lack of "curly" wings indicating desired genes were passed on) were then separated and placed in a 29 ℃ incubator to activate the knockdown system. After 5-6 days in the incubator, flies were infected with *Pe* by administering 200μL of *Pe* solution directly into the vial and by placing a paste made from the remaining *Pe* solution and ground yeast along the sides of the vial. Circular pieces of paper were placed in the vial beforehand to absorb the *Pe* culture and prevent flies from drowning in liquid. After infection, flies were left in a 29 ℃ incubator for 18 hours before being removed and dissected.

Fly guts were dissected using a stereo microscope in a dish containing 1x phosphate buffered saline (PBS). The dissection protocol was as follows: first, remove the head of the fly. Next,

remove the end of the rectum which will begin pulling the gut outside of the thorax. Pull until a small amount the gut is exposed and then separate the thorax from the abdomen. This should reveal the crop, a

relatively large and white organ, which can be pulled to dislodge the gut from the abdomen. Lastly, remove the crop, slide the thorax off the gut, and remove connective tissue and organs (such as the ovaries).

C. Imaging Protocol

The dissected guts were placed into labeled centrifuge tubes and kept on ice while dissections were finished. Immediately after dissections were finished, the guts were "fixed" by adding a 16% paraformaldehyde (PFA) in PBS solution until the final solution contained 4% PFA. Guts were kept in 4% PFA solution for 25-30 minutes.

After fixation, "washes" were performed on the guts. Washing protocol consisted of removing as much liquid as possible from the tubes with a pipette and refilling the tube with a 0.2% Triton X-100 in PBS solution (0.2% PBST). Three long and three short washes were performed in between each stage of staining. Long washes consisted of placing the guts in 0.2 PBST on a rotating mixer for 15 minutes before proceeding to the next wash. Short washes consisted of removing and refilling the tube with solution without wait time. In this way, the guts in PFA were "washed" and then 10% goat serum in 0.2% PBST was added to the tubes and mixed for 25-30 minutes. This solution of goat serum acted as a blocking buffer to increase the fidelity of the subsequent immunostaining. A 1:1000 dilution of primary antibodies in PBST was then added to the tubes and the tubes were placed in a 4℃ fridge for 40-50 hours. After this time, guts were washed and a 1:1000 dilution of secondary

antibodies was added to the tubes. Tubes were placed on a rotating mixer for 1.5-2 hours before guts were washed again.

Once immunostaining had been completed, guts were arranged in rows on a microscope slide with a drop of VECTASHIELD and several drops of 1x PBS. Cover slips were secured over the guts on the slides using nail polish. Slides were then labeled and ready for quantification and analysis.

D. Quantification of Dividing Cells and Data Analysis

Fluorescence microscopy was used to visualize and analyze prepared slides. pH3 positive cells were manually counted in each fly gut. Once all cells had been counted, data were input into GraphPad Prism, a data analysis software from the company Dotmatics. Unpaired T-tests were performed between the control and each RNAi line that had been prepared in that round of screening. Using the results from these tests, genes corresponding to the RNAi lines were classified as low count or high count if the p-value was less than 0.05. Low count genes, when knocked down, caused a lower amount of pH3 positive cells to be seen while high count genes increased the amount of pH3 positive cells.

IV. RESULTS

A. RNAi Screen Identified 28 High and 14 Low Count Genes

There were 82 total genes screened in this project and 42 were identified as significantly involved in ISC proliferation. As discussed in the methods section, genes were only considered significant if a t-test between the specific gene and a control comparing the amount of cell proliferation had a p-value of less than 0.05. Twelve different rounds of screening including 5-10 genes and a control were performed (see Figure 1 for an example of data collected in a batch of screening).

The several rounds of screening identified 28 genes whose knockdown led to increased cell proliferation (high count

genes), 13 genes whose knockdown decreased cell proliferation

(low count genes), and 42 genes whose knockdown had no significant effect on cell proliferation. Results were

organized into tables that each contain the following information: the RNAi number used to identify fly lines within the lab, the common name of the gene targeted for knockdown, and the stock ID number for the fly library where the stock was obtained (see tables 1, 2, and 3 below).

Figure 1 – The number of dividing ISC cells were counted for each gut and RNAi line before being compared to the control (w1118) in an unpaired t-test to identify significant differences. The x-axis shows the gene number used to identify each fly line in the lab and the y-axis shows the number of dividing cells (pH3 cells). Each dot represents the dividing cell count seen in one gut for a particular gene. This batch showed gene 347 significantly increased cell proliferation and 469 decreased it (p-value<0.05).

Genes' Effect on ISC Proliferation
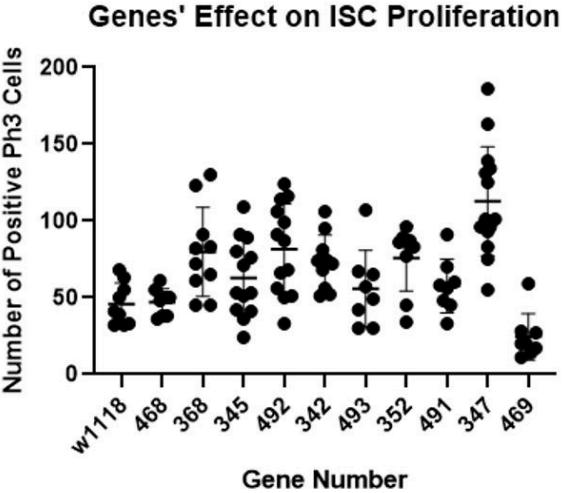
Table 1: High Count Genes

RNAi #	Targeted Gene	Stock ID #	Library
44	CG10916	107518	VDRC
68-2	CG13654	13550	VDRC
99	CG1698	101947	VDRC
103	CG17599	14682	VDRC
104	CG17919	38204	VDRC
105	CG17982	33033	VDRC
166	CG4393	105381	VDRC
175	CG5550	103982	VDRC
203	CG8353	105454	VDRC
215	CG30293	108476	VDRC
277	GstD5	102234	VDRC
284	ImpL2	30930	VDRC
284-2	ImpL2	106543	VDRC
289	JhI-1	110285	VDRC
290	Jra	107997	VDRC
293	Kay	33379	TRiP
331	nst	105398	VDRC
332	nub	105044	VDRC
338	Oseg6	38462	VDRC

Table 2: Low Count Genes

RNAi #	Targeted Gene	Stock ID #	Library
39	CG10183	105599	VDRC
44-2	CG10916	31379	VDRC
84	CG15043	23066	VDRC
96	CG1599	108733	VDRC

97	CG1628	109588	VDRC
197	CG7806	38997	TRiP
271	Gli	107258	VDRC
309	Marf	31157	TriP
313	Mkp3	45415	VDRC
333	Nup153	107750	VDRC
339	Oseg6	43263	TriP
367	Rtnl1	110545	VDRC
370	scu	110802	VDRC
469	dx	106086	VDRC

Table 3: Not Significant Genes

RNAi #	Targeted Gene	Stock ID #	Library
52	CG12112	104224	VDRC
55	CG12520	107605	VDRC
68	CG13654	110349	VDRC
73	CG14218	102532	VDRC
76	CG14339	17493	VDRC
84-2	CG15043	23067	VDRC
98	CG16721	103729	VDRC
102	CG17564	47273	VDRC
178-3	CG5656	18119	VDRC
178	CG5656	110733	VDRC

311	Mekk1	110339	VDRC
312	Mkk4	108561	VDRC
315	mnd	110217	VDRC
327	nAcRbeta-21C	101868	VDRC
329	Nmda1	108378	VDRC
330	Npl4	109309	VDRC
334	Nup44A	106489	VDRC
335	Ocrl	110796	VDRC
337	Orct2	106681	VDRC
341	pcl	108271	VDRC
345	PGRP-LA	102277	VDRC
366	rtGEF	100583	VDRC
369	scb	100949	VDRC
424	zip	38259	TRiP
428	CG2991	2604	VDRC
431	CG33116	9308	VDRC
449	CG6290	35600	VDRC
455	CG33468	48414	VDRC
468	CG9925	106278	VDRC
491	STAT92E	33637	TRIP
493	CG14340	52620	VDRC

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V. DISCUSSION

Colorectal cancer is the second most common cause of cancer-related deaths in the world, with approximately 1.8 million new cases each year [1]. To better understand mechanisms of cell division and growth in colorectal cancer, this project aimed to identify which genes in the gut are responsible for sensing damage and activating cell proliferation. Disabling a gene suspected in cell proliferation pathways and quantifying the effect on cell proliferation is a proven method for studying genes. In this project, genes were disabled and studied by performing an RNAi gene knockdown screen using *Drosophila melanogaster* (i.e., fruit flies). Of the 82 genes that went through the screen, 42 were identified as significant in regulating cell proliferation. To inhibit colorectal cancer cell's ability to grow and spread, future treatments could potentially target any of the 42 identified genes. Though more work is necessary to develop such treatments, these results lay the foundation for more effective treatments that will reduce the worldwide impact of colorectal cancer.

Knockdown or silencing of the 42 identified genes in the screen caused cell proliferation to either increase or decrease (see Figure 1). Since the fly intestines were infected with bacteria and damaged as part of the screening process, it is likely that the 42 identified genes play some role in regulating damage-mediated cell proliferation. The 14 "low count genes" likely promote or upregulate cell proliferation in response to damage (see Table 2). The 28 "high count genes" likely do the opposite; inhibit or downregulate damage-mediated cell proliferation (see Table 1).

Many of the potential colorectal oncogenes identified in this project have orthologs in humans that could potentially be

targeted for cancer treatment. For example, For example, the "Marf" gene (see Table 2) has a DIOPT score of 13 out of 19 with the human gene MFN2 [65], [66]. This score corresponds to the level of consensus among gene databases and scientific papers that the two genes are indeed orthologs. This relatively high score indicates that MFN2 is possibly involved in intestinal damage-sensing and cell proliferation pathways as Marf is in flies. MFN2 has already shown to be involved in cell proliferation elsewhere in the body, which supports that its identification as a potentially significant gene in intestinal cell renewal was likely accurate [67]. Many other genes identified in this screen have DIOPT scores as high as 15,

with several of human orthologs of genes such as "scu" and "Oseg6" (see Table 2) not having previously been implicated in cell proliferation pathways. Any one of these orthologs could be furthered studied to lead to potential new colorectal cancer treatments.

The two main limitations of this screening project were the inability implement techniques for mitigating off-target effects and sample sizes of five or less for certain genes. It is known that RNAi screens can have false positives due to off-target effects of the siRNAs where genes other than the targeted one are silenced or affected [68]. Though several techniques exist to mitigate such off-target effects, they were could not feasibly be implemented in this project [69]. Second, due to rare instances of fly mishandling or premature fly death, some lines were screen with relatively small sample sizes (<5).

Despite project limitations, it is still likely that the 42 identified genes could serve as potential cancer therapy targets. For example, look at the case of the long-studied ADAR1 gene. Though previously only implicated as a potential target for cancer treatment, recent studies that better elucidated its

mechanisms have allowed researchers to begin formulating ideas for new cancer treatments that would target this gene [70]. Though it is possible that many of the 42 identified are not feasible targets, these results allow researchers to focus their efforts on a smaller pool of potential targets rather than making educated guesses on which genes to start with. This could save large amounts of time and money from being spent on fruitless projects, which can then be reallocated to more promising ones. Ideally, this means that better cancer treatments are discovered quicker and made available to patients sooner.

Future work for this project entails performing a secondary screen on the "high count genes" and possibly re-screening genes that had low sample sizes. The secondary screen on the high-count genes will be performed without a bacterial infection. If knockdown of one of these genes still results in increased cell proliferation despite the intestine not facing damage or stress due to bacterial toxins, it is likely those genes are not involved in damage-mediated cell proliferation. However, if knockdown of a given high-count gene without infection shows similar cell division to that of the control, then it would be very likely that the gene does play a role in damage-mediated cell proliferation. Future studies are being considered to explore the function of the genes that caused the greatest changes in cell proliferation, but are still in planning stages at this moment.

Colorectal cancer continues to be one of the world's most prevalent and deadly diseases [1]. Recent studies seem to suggest that its impact on younger and younger generations will increase in the coming years [71], [72]. Current treatment options for colorectal cancer, such as chemotherapy and surgery, are less than optimal as they often harm healthy cells as well as cancer cells [73], [74]. Though the results of this

screening project do not immediately benefit colorectal cancer patients, they do lay the foundation for future studies and improved cancer therapies that could vastly improve colorectal cancer treatment options. These optimized treatments could be more affordable, less damaging to healthy cells, and lead to improved patient outcomes. Such treatments would dramatically reduce the global financial burden caused by colorectal cancer and, most importantly, would help to reduce the nearly 1 million deaths that it causes each year [1].

REFERENCES

[1] H. Sung *et al.*, "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *CA. Cancer J. Clin.*, vol. 71, no. 3, pp. 209–249, May 2021, doi: 10.3322/caac.21660.

[2] R. J. Duronio and Y. Xiong, "Signaling Pathways that Control Cell Proliferation," *Cold Spring Harb. Perspect. Biol.*, vol. 5, no. 3, pp. a008904–a008904, Mar. 2013, doi: 10.1101/cshperspect.a008904.

[3] W. M. Wong and N. A. Wright, "Cell proliferation in gastrointestinal mucosa," *J. Clin. Pathol.*, vol. 52, no. 5, pp. 321–333, May 1999, doi: 10.1136/jcp.52.5.321.

[4] C. Crosnier, D. Stamataki, and J. Lewis, "Organizing cell renewal in the intestine: stem cells, signals and combinatorial control," *Nat. Rev. Genet.*, vol. 7, no. 5, pp. 349–359, May 2006, doi: 10.1038/nrg1840.

[5] B. K. Staley and K. D. Irvine, "Warts and Yorkie Mediate Intestinal Regeneration by Influencing Stem Cell Proliferation," *Curr. Biol.*, vol. 20, no. 17, pp. 1580–1587, Sep. 2010, doi: 10.1016/j.cub.2010.07.041.

[6] C. Andrews, M. H. McLean, and S. K. Durum, "Cytokine Tuning of Intestinal Epithelial Function," *Front. Immunol.*, vol. 9, p. 1270, Jun. 2018, doi: 10.3389/fimmu.2018.01270.

[7] P. Sansone and J. Bromberg, "Targeting the Interleukin-6/ Jak/Stat Pathway in Human Malignancies," *J. Clin. Oncol.*, vol. 30, no. 9, pp. 1005–1014, Mar. 2012, doi: 10.1200/ JCO.2010.31.8907.

[8] A. Casali and E. Batlle, "Intestinal Stem Cells in Mammals and Drosophila," *Cell Stem Cell*, vol. 4, no. 2, pp. 124–127, Feb. 2009, doi: 10.1016/j.stem.2009.01.009.

[9] M. Krausova and V. Korinek, "Wnt signaling in adult intestinal stem cells and cancer," *Cell. Signal.*, vol. 26, no. 3, pp. 570–579, Mar. 2014, doi: 10.1016/j.cellsig.2013.11.032.

[10] E. W. Sayers *et al.*, "Database resources of the national center for biotechnology information," *Nucleic Acids Res.*, vol. 50, no. D1, pp. D20–D26, Jan. 2022, doi: 10.1093/nar/gkab1112.

[11] H. Jiang, P. H. Patel, A. Kohlmaier, M. O. Grenley, D. G. McEwen, and B. A. Edgar, "Cytokine/Jak/Stat Signaling Mediates Regeneration and Homeostasis in the Drosophila Midgut," *Cell*, vol. 137, no. 7, pp. 1343–1355, Jun. 2009, doi: 10.1016/j.cell.2009.05.014.

[12] P. H. Patel *et al.*, "Damage sensing by a Nox-Ask1-MKK3-p38 signaling pathway mediates regeneration in the adult Drosophila midgut," *Nat. Commun.*, vol. 10, no. 1, p. 4365, Dec. 2019, doi: 10.1038/s41467-019-12336-w.

[13] H. Jiang, A. Tian, and J. Jiang, "Intestinal stem cell response to injury: lessons from Drosophila," *Cell. Mol. Life Sci.*, vol. 73, no. 17, pp. 3337–3349, Sep. 2016, doi: 10.1007/ s00018-016-2235-9.

[14] T. Jess *et al.*, "Risk of Intestinal Cancer in Inflammatory Bowel Disease: A Population-Based Study From Olmsted County, Minnesota," *Gastroenterology*, vol. 130, no. 4, pp. 1039–1046, Apr. 2006, doi: 10.1053/j.gastro.2005.12.037.

[15] D. R. Green, "Cell Death and Cancer," *Cold Spring Harb. Perspect. Biol.*, vol. 14, no. 9, p. a041103, Sep. 2022, doi:

10.1101/cshperspect.a041103.

[16] J. C. Reed, "Mechanisms of Apoptosis," *Am. J. Pathol.*, vol. 157, no. 5, pp. 1415–1430, Nov. 2000, doi: 10.1016/S0002-9440(10)64779-7.

[17] J. Inagaki, V. Rodriguez, and G. P. Bodey, "Causes of death in cancer patients," *Cancer*, vol. 33, no. 2, pp. 568–573, Feb. 1974, doi: 10.1002/1097-0142(197402)33:2<568::AID-CNCR2820330236>3.0.CO;2-2.

[18] C. Franceschi, "Cell proliferation, cell death and aging," *Aging Clin. Exp. Res.*, vol. 1, no. 1, pp. 3–15, Sep. 1989, doi: 10.1007/BF03323871.

[19] K. Vermeulen, D. R. Van Bockstaele, and Z. N. Berneman, "The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer: *Cell cycle regulation and deregulation,*" *Cell Prolif.*, vol. 36, no. 3, pp. 131–149, Jun. 2003, doi: 10.1046/j.1365-2184.2003.00266.x.

[20] T. K. Noah and N. F. Shroyer, "Notch in the Intestine: Regulation of Homeostasis and Pathogenesis," *Annu. Rev. Physiol.*, vol. 75, no. 1, pp. 263–288, Feb. 2013, doi: 10.1146/annurev-physiol-030212-183741.

[21] J. Y. Ong and J. Z. Torres, "Dissecting the mechanisms of cell division," *J. Biol. Chem.*, vol. 294, no. 30, pp. 11382–11390, Jul. 2019, doi: 10.1074/jbc.AW119.008149.

[22] L. Ding *et al.*, "The Roles of Cyclin-Dependent Kinases in Cell-Cycle Progression and Therapeutic Strategies in Human Breast Cancer," *Int. J. Mol. Sci.*, vol. 21, no. 6, p. 1960, Mar. 2020, doi: 10.3390/ijms21061960.

[23] J. Wade Harper and S. J. Elledge, "Cdk inhibitors in development and cancer," *Curr. Opin. Genet. Dev.*, vol. 6, no. 1, pp. 56–64, Feb. 1996, doi: 10.1016/S0959-437X(96)90011-8.

[24] R. W. Clapp, M. M. Jacobs, and E. L. Loechler, "Environmental and Occupational Causes of Cancer: New

Evidence 2005-2007," *Rev. Environ. Health*, vol. 23, no. 1, pp. 1–38, Jan. 2008, doi: 10.1515/REVEH.2008.23.1.1.

[25] E. J. Davies, V. Marsh, and A. R. Clarke, "Origin and maintenance of the intestinal cancer stem cell: ORIGIN AND MAINTENANCE OF THE ICSC," *Mol. Carcinog.*, vol. 50, no. 4, pp. 254–263, Apr. 2011, doi: 10.1002/mc.20631.

[26] O. Kiraly, G. Gong, W. Olipitz, S. Muthupalani, and B. P. Engelward, "Inflammation-Induced Cell Proliferation Potentiates DNA Damage-Induced Mutations In Vivo," *PLOS Genet.*, vol. 11, no. 2, p. e1004901, Feb. 2015, doi: 10.1371/journal.pgen.1004901.

[27] T. A. Ullman and S. H. Itzkowitz, "Intestinal Inflammation and Cancer," *Gastroenterology*, vol. 140, no. 6, pp. 1807-1816.e1, May 2011, doi: 10.1053/j.gastro.2011.01.057.

[28] F. Radtke and H. Clevers, "Self-Renewal and Cancer of the Gut: Two Sides of a Coin," *Science*, vol. 307, no. 5717, pp. 1904–1909, Mar. 2005, doi: 10.1126/science.1104815.

[29] F. Radtke, H. Clevers, and O. Riccio, "From Gut Homeostasis to Cancer," *Curr. Mol. Med.*, vol. 6, no. 3, pp. 275–289, May 2006, doi: 10.2174/156652406776894527.

[30] N. Barker *et al.*, "Crypt stem cells as the cells-of-origin of intestinal cancer," *Nature*, vol. 457, no. 7229, pp. 608–611, Jan. 2009, doi: 10.1038/nature07602.

[31] F. J. Abdul Khalek, G. I. Gallicano, and L. Mishra, "Colon cancer stem cells," *Gastrointest. Cancer Res. GCR*, no. Suppl 1, pp. S16-23, Nov. 2010.

[32] K. Chen, Y. Huang, and J. Chen, "Understanding and targeting cancer stem cells: therapeutic implications and challenges," *Acta Pharmacol. Sin.*, vol. 34, no. 6, pp. 732–740, Jun. 2013, doi: 10.1038/aps.2013.27.

[33] M. M. Olsen, K. B. LeFebvre, K. J. Brassil, and Oncology Nursing Society, Eds., *Chemotherapy and immunotherapy*

guidelines and recommendations for practice. Pittsburgh, Pennsylvania: Oncology Nursing Society, 2019.

[34] B. Gustavsson *et al.*, "A Review of the Evolution of Systemic Chemotherapy in the Management of Colorectal Cancer," *Clin. Colorectal Cancer*, vol. 14, no. 1, pp. 1–10, Mar. 2015, doi: 10.1016/j.clcc.2014.11.002.

[35] D. B. Longley, D. P. Harkin, and P. G. Johnston, "5-Fluorouracil: mechanisms of action and clinical strategies," *Nat. Rev. Cancer*, vol. 3, no. 5, pp. 330–338, May 2003, doi: 10.1038/nrc1074.

[36] N. Petrelli *et al.*, "A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma.," *J. Clin. Oncol.*, vol. 5, no. 10, pp. 1559–1565, Oct. 1987, doi: 10.1200/JCO.1987.5.10.1559.

[37] S. Waxman and H. Bruckner, "The enhancement of 5-fluorouracil antimetabolic activity by leucovorin, menadione and α-tocopherol," *Eur. J. Cancer Clin. Oncol.*, vol. 18, no. 7, pp. 685–692, Jul. 1982, doi: 10.1016/0277-5379(82)90215-2.

[38] C. M. Stoscheck and L. E. King Jr., "Role of Epidermal Growth Factor in Carcinogenesis," *Cancer Res.*, vol. 46, no. 3, pp. 1030–1037, Mar. 1986.

[39] Y. Imai, C. K. H. Leung, H. G. Friesen, and R. P. C. Shiu, "Epidermal Growth Factor Receptors and Effect of Epidermal Growth Factor on Growth of Human Breast Cancer Cells in Long-Term Tissue Culture1," *Cancer Res.*, vol. 42, no. 11, pp. 4394–4398, Nov. 1982.

[40] C. Tofthagen, "Surviving Chemotherapy for Colon Cancer and Living with the Consequences," *J. Palliat. Med.*, vol. 13, no. 11, pp. 1389–1391, Nov. 2010, doi: 10.1089/jpm.2010.0124.

[41] E. P. M. de Almeida, M. G. R. de Gutiérrez, and N. P. Adami, "Monitoramento e avaliação dos efeitos colaterais da quimioterapia em pacientes com câncer de cólon," *Rev. Lat. Am. Enfermagem*, vol. 12, no. 5, pp. 760–766, Oct. 2004, doi: 10.1590/S0104-11692004000500009.

[42] QUASAR Collaborative Group, "Adjuvant chemotherapy versus observation in patients with colorectal cancer: a

randomised study," *The Lancet*, vol. 370, no. 9604, pp. 2020–2029, Dec. 2007, doi: 10.1016/S0140-6736(07)61866-2.

[43] P. M. Wigmore, S. Mustafa, M. El-Beltagy, L. Lyons, J. Umka, and G. Bennett, "Effects of 5-FU," in *Chemo Fog*, vol. 678, R. B. Raffa and R. J. Tallarida, Eds. New York, NY: Springer New York, 2010, pp. 157–164. doi: 10.1007/978-1-4419-6306-2_20.

[44] T. Lan, H. Que, M. Luo, X. Zhao, and X. Wei, "Genome editing via non-viral delivery platforms: current progress in personalized cancer therapy," *Mol. Cancer*, vol. 21, no. 1, p. 71, Mar. 2022, doi: 10.1186/s12943-022-01550-8.

[45] X. Lai *et al.*, "A scalable solver for a stochastic, hybrid cellular automaton model of personalized breast cancer therapy," *Int. J. Numer. Methods Biomed. Eng.*, vol. 38, no. 1, Jan. 2022, doi: 10.1002/cnm.3542.

[46] C. Rodríguez-Antona and M. Taron, "Pharmacogenomic biomarkers for personalized cancer treatment," *J. Intern. Med.*, vol. 277, no. 2, pp. 201–217, Feb. 2015, doi: 10.1111/joim.12321.

[47] K. G. Samsom *et al.*, "Study protocol: Whole genome sequencing Implementation in standard Diagnostics for Every cancer patient (WIDE)," *BMC Med. Genomics*, vol. 13, no. 1, p. 169, Dec. 2020, doi: 10.1186/s12920-020-00814-w.

[48] K. Schwarze, J. Buchanan, J. C. Taylor, and S. Wordsworth, "Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of

the literature," *Genet. Med.*, vol. 20, no. 10, pp. 1122–1130, Oct. 2018, doi: 10.1038/gim.2017.247.

[49] C. S. Shemesh *et al.*, "Personalized Cancer Vaccines: Clinical Landscape, Challenges, and Opportunities," *Mol. Ther.*, vol. 29, no. 2, pp. 555–570, Feb. 2021, doi: 10.1016/ j.ymthe.2020.09.038.

[50] A. Burguin, C. Diorio, and F. Durocher, "Breast Cancer Treatments: Updates and New Challenges," *J. Pers. Med.*, vol. 11, no. 8, p. 808, Aug. 2021, doi: 10.3390/jpm11080808.

[51] Z. Mirzoyan, M. Sollazzo, M. Allocca, A. M. Valenza, D. Grifoni, and P. Bellosta, "Drosophila melanogaster: A Model Organism to Study Cancer," *Front. Genet.*, vol. 10, p. 51, Mar. 2019, doi: 10.3389/fgene.2019.00051.

[52] B. Ugur, K. Chen, and H. J. Bellen, "*Drosophila* tools and assays for the study of human diseases," *Dis. Model. Mech.*, vol. 9, no. 3, pp. 235–244, Mar. 2016, doi: 10.1242/dmm.023762.

[53] Y. Apidianakis and L. G. Rahme, "*Drosophila melanogaster* as a model for human intestinal infection and pathology," *Dis. Model. Mech.*, vol. 4, no. 1, pp. 21–30, Jan. 2011, doi: 10.1242/dmm.003970.

[54] M. Yamaguchi and H. Yoshida, "Drosophila as a Model Organism," in *Drosophila Models for Human Diseases*, vol. 1076, M. Yamaguchi, Ed. Singapore: Springer Singapore, 2018, pp. 1–10. doi: 10.1007/978-981-13-0529-0_1.

[55] K. G. Hales, C. A. Korey, A. M. Larracuente, and D. M. Roberts, "Genetics on the Fly: A Primer on the *Drosophila* Model System," *Genetics*, vol. 201, no. 3, pp. 815–842, Nov. 2015, doi: 10.1534/genetics.115.183392.

[56] R. C. Wilson and J. A. Doudna, "Molecular Mechanisms of RNA Interference," *Annu. Rev. Biophys.*, vol. 42, no. 1, pp. 217–239, May 2013, doi: 10.1146/annurev-biophys-083012-130404.

[57] N. Perrimon, J.-Q. Ni, and L. Perkins, "In vivo RNAi: Today and Tomorrow," *Cold Spring Harb. Perspect. Biol.*, vol. 2, no. 8, pp. a003640–a003640, Aug. 2010, doi: 10.1101/cshperspect.a003640.

[58] M. L. Suster, L. Seugnet, M. Bate, and M. B. Sokolowski, "Refining GAL4-driven transgene expression inDrosophila with a GAL80 enhancer-trap," *genesis*, vol. 39, no. 4, pp. 240–245, Aug. 2004, doi: 10.1002/gene.20051.

[59] A. H. Brand and N. Perrimon, "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes," *Development*, vol. 118, no. 2, pp. 401–415, Jun. 1993, doi: 10.1242/dev.118.2.401.

[60] S. E. McGuire, P. T. Le, A. J. Osborn, K. Matsumoto, and R. L. Davis, "Spatiotemporal Rescue of Memory Dysfunction in *Drosophila*," *Science*, vol. 302, no. 5651, pp. 1765–1768, Dec. 2003, doi: 10.1126/science.1089035.

[61] F. D. Sigoillot and R. W. King, "Vigilance and Validation: Keys to Success in RNAi Screening," *ACS Chem. Biol.*, vol. 6, no. 1, pp. 47–60, Jan. 2011, doi: 10.1021/cb100358f.

[62] S. E. Mohr, J. A. Smith, C. E. Shamu, R. A. Neumüller, and N. Perrimon, "RNAi screening comes of age: improved techniques and complementary approaches," *Nat. Rev. Mol. Cell Biol.*, vol. 15, no. 9, pp. 591–600, Sep. 2014, doi: 10.1038/nrm3860.

[63] Z. Zhai *et al.*, "Accumulation of differentiating intestinal stem cell progenies drives tumorigenesis," *Nat. Commun.*, vol. 6, no. 1, p. 10219, Dec. 2015, doi: 10.1038/ncomms10219.

[64] D. E. Miller, K. R. Cook, and R. S. Hawley, "The joy of balancers," *PLOS Genet.*, vol. 15, no. 11, p. e1008421, Nov. 2019, doi: 10.1371/journal.pgen.1008421.

[65] K. Clark, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and

E. W. Sayers, "GenBank," *Nucleic Acids Res.*, vol. 44, no. D1, pp. D67–D72, Jan. 2016, doi: 10.1093/nar/gkv1276.

[66] Y. Hu *et al.*, "FlyRNAi.org—the database of the Drosophila RNAi screening center and transgenic RNAi project: 2021 update," *Nucleic Acids Res.*, vol. 49, no. D1, pp. D908–D915, Jan. 2021, doi: 10.1093/nar/gkaa936.

[67] P. Zanfardino and V. Petruzzella, "Autophagy and proliferation are dysregulated in Charcot-Marie-Tooth disease type 2A cells harboring MFN2 (mitofusin 2) mutation," *Autophagy Rep.*, vol. 1, no. 1, pp. 537–541, Dec. 2022, doi: 10.1080/27694127.2022.2132447.

[68] N. Schultz *et al.*, "Off-target effects dominate a large-scale RNAi screen for modulators of the TGF-β pathway and reveal microRNA regulation of TGFBR2," *Silence*, vol. 2, no. 1, p. 3, 2011, doi: 10.1186/1758-907X-2-3.

[69] A. L. Jackson and P. S. Linsley, "Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application," *Nat. Rev. Drug Discov.*, vol. 9, no. 1, pp. 57–67, Jan. 2010, doi: 10.1038/nrd3010.

[70] A. R. Baker and F. J. Slack, "ADAR1 and its implications in cancer development and treatment," *Trends Genet.*, vol. 38, no.

8, pp. 821–830, Aug. 2022, doi: 10.1016/j.tig.2022.03.013.

[71] E. Feletto *et al.*, "Trends in Colon and Rectal Cancer Incidence in Australia from 1982 to 2014: Analysis of Data on Over 375,000 Cases," *Cancer Epidemiol. Biomarkers Prev.*, vol. 28, no. 1, pp. 83–90, Jan. 2019, doi: 10.1158/1055-9965.EPI-18-0523.

[72] R. L. Siegel, C. D. Jakubowski, S. A. Fedewa, A. Davis, and N. S. Azad, "Colorectal Cancer in the Young: Epidemiology, Prevention, Management," *Am. Soc. Clin. Oncol. Educ. Book*, no. 40, pp. e75–e88, May 2020, doi: 10.1200/EDBK_279901.

[73] S. M. Koroukian, F. Xu, P. M. Bakaki, M. Diaz-Insua, T. P.

Towe, and C. Owusu, "Comorbidities, Functional Limitations, and Geriatric Syndromes in Relation to Treatment and Survival Patterns Among Elders With Colorectal Cancer," *J. Gerontol. A. Biol. Sci. Med. Sci.*, vol. 65A, no. 3, pp. 322–329, Mar. 2010, doi: 10.1093/gerona/glp180.

[74] D. Symeonidis, G. Christodoulidis, G. Koukoulis, M. Spyridakis, and K. Tepetes, "Colorectal cancer surgery in the elderly: limitations and drawbacks," *Tech. Coloproctology*, vol. 15, no. S1, pp. 47–50, Oct. 2011, doi: 10.1007/s10151-011-0751-z.

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70. Research Reflection by Carter Niedert

Carter Niedert

Faculty Mentor: Bruce Edgar (Oncological Sciences, University of Utah)

My undergraduate research experience has been amazing! I have been exposed to so many brilliant individuals and ideas that have enriched my college experience. I have learned numerous techniques from immunofluorescent staining and imaging to gel electrophoresis and so much more. I hope to become a physician one day and I know that these experiences will enhance my ability to perform research as a medical professional. I know that these experiences have given me the tools to launch a successful, lifelong journey into the world of research!

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71.

Characterization of Satellite-Derived Air Pollutant Measurements for T2DM Incidence Correlation Studies

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Faculty Mentor: Ramkiran Gouripeddi (Biomedical Informatics, University of Utah)

Introduction: Physiologically detrimental chemicals from motor vehicles, fossil fuels, and industrial emissions can have devastating impacts on global health. Chemical pollution and particulates can be inhaled deep into the lungs and enter circulation within the bloodstream, contributing to tissue damage, increased cardiovascular or respiratory health risks, and inflammatory or metabolic insults [1]. Preliminary studies have shown that criteria pollutants (CO, NO2, O3, PM2.5, SO2) stimulate these conditions, potentially promoting an increase in type 2 diabetes mellitus (T2DM) incidence in locations with adverse air quality (AQ) [2], inducing lifelong morbidity and encumbering the health care system. Forthcoming economic development and urbanization cause projected increases in air pollution and an increased burden of T2DM incidence [1], demonstrating the need for widespread and accurate pollutant measurements. Several studies have utilized ground monitored AQ measurements to investigate the correlation between T2DM prevalence and exposure levels, finding statistically significant, positive relationships [1,2]. Unfortunately, not all AQ and T2DM relationship factors are reported consistently: such studies still hold considerable ambiguity concerning the validity of the ground-monitor obtained AQ measurements. A

critical issue not successfully addressed is the reliability of a ground based AQ monitor on those living outside its range. Ground-based monitors are generally located in population-dense areas, leaving 79 million Americans in unmonitored or monitor sparse locations [3]. In contrast, satellite data from the National Aeronautics and Space Administration (NASA) gives access to spatiotemporal air pollution data through global exposure coverage of criteria pollutant concentrations through geographic coordinate specific values.

Methods: To expand the spatial and temporal availability of air quality data, a data retrieval infrastructure that generated practical, spatially, and temporally widespread chemical pollutant files for the criteria pollutants separated by year and location was developed. This infrastructure was designed through NASA's Modern-Era Retrospective analysis for Research and Applications, Version 2 (MERRA-2) and Ozone Monitoring Instrument (OMI) pollutant concentration measurements [4], available through geographic-coordinate gridded daily files. After these daily files were retrieved for each of the criteria pollutants, they were filtered for missing days and quality flags that may arise in heavy cloud or snow conditions. The gridded data was matched to the zip code and census tract level using reverse geocoding with parallelized K-D tree implementation. This NASA satellite data retrieval infrastructure transformed 30 thousand daily chemical pollution files into a data resource with 15 years of criteria pollutant concentration data, organized by year and location with indicators on data missingness and quality.

Figure 1. (a) Utah County Environmental Protection Agency, (b) Utah County MERRA-2 Satellite, (c) Carbon County MERRA-2 Satellite 2019 PM2.5 Data Trends.
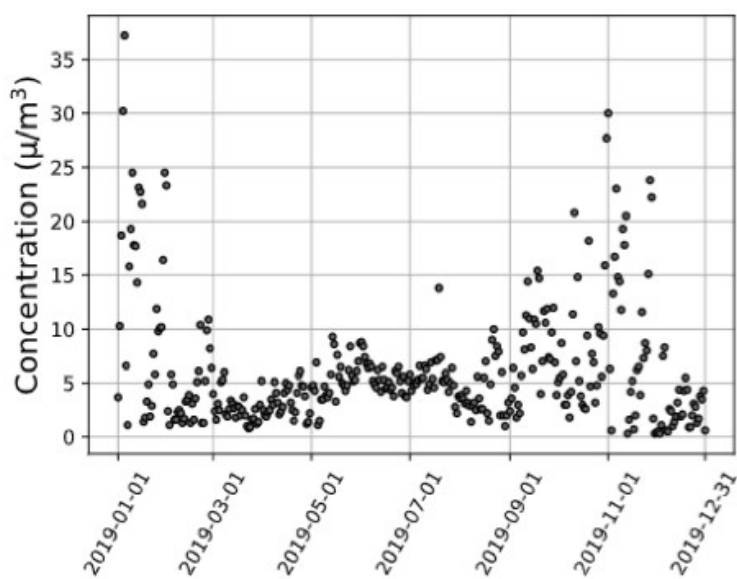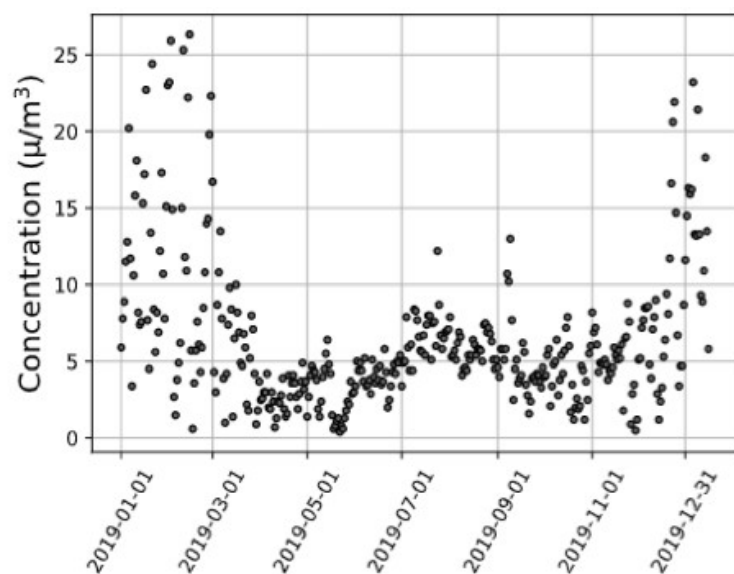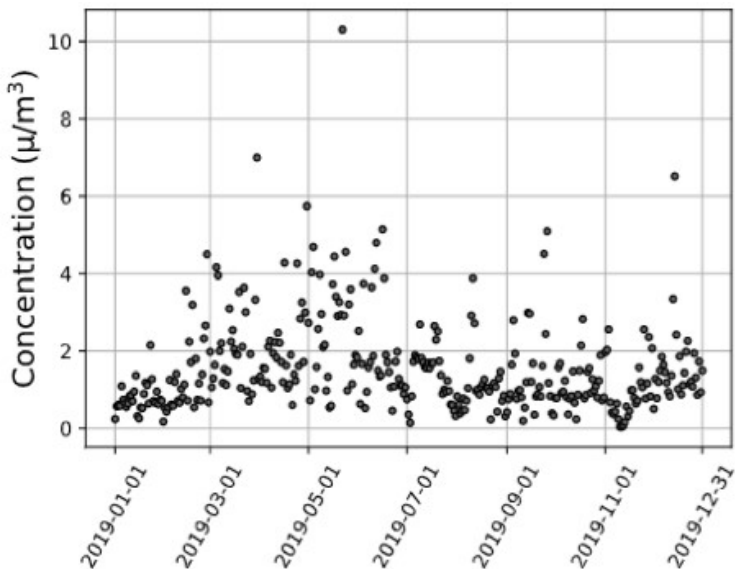
Discussion: With the results of this analysis, satellite data could be used to track pollutant profiles, provide support for AQ forecasting, and provide input to AQ models and data for model evaluation. For example, the closest ground-based monitor air quality monitor to Carbon County, Utah, is 145 kilometers away, located in Utah County. As demonstrated in Figure 1, although the Utah County satellite and Utah County Environmental Protection Agency (EPA) monitor measurements model one another, there are significant differences between the EPA and satellite Utah County measurements and the satellite Carbon County measurements. This variation between satellite instrument AQ measurements and EPA monitor measurements quantified through a correlation quantification exemplifies a county where satellite AQ data could provide more precise criteria pollutant concentrations. This illustrates how satellite data have considerable applications in locations lacking AQ monitors or complement data from sites with existing ground monitors.

Our study will provide insight into future satellite-health applications, emphasizing satellite data with ground monitors to advance knowledge on chemical pollutants' contribution to cardiometabolic disease morbidity. Correspondingly, suitable public health legislative policy regarding AQ could be created abreast of reliable, widespread pollutant exposure levels.

References: [1] Yang A. *et al. Environmental International (2021).* [2] Riches N. *et al. Environmental Research. (2022).* [3] US Environmental Protection Agency. Pre-generated data files. Accessed July 13, 2020. https://aqs.epa.gov/aqsweb/airdata/ download_files.html. [4] NASA MERRA-2 and OMI. Daily data files. Accessed June 10, 2020. https://disc.gsfc.nasa.gov/ datasets.

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72. Research Reflection by Adriana Payan-Medina

Adriana Payan-Medina

Through my undergraduate chemical engineering degree, I have prioritized the development of research skills that will position me to formulate hypotheses and evaluate the relationships between changes in exposomic conditions and human health. This has allowed me to contribute to the toxicology field as a co-author in Environmental Research with a first authorship in preparation (see proposed research summary) and as a scientific presenter at 11 regional, national, and international conferences. I've become immersed in research through the University of Utah Summer Program for Undergraduate Research, the UCLA Bruins-in-Genomics NSF

REU, and the Broad Institute of MIT and Harvard Summer Research Program. Due to my research accomplishments in these roles, I was recognized as a 2021 NIH Research Fellow and a 2022 Barry Goldwater scholar. Through my Ph.D. in bioengineering, I aspire to advance medicine by developing improved culture models for environmental toxicology studies. I ultimately aspire to become a professor and hope to use my platform to inspire racial-ethnic minorities, women, and rural students to pursue education in engineering by fostering an inclusive and supportive academic environment.

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73.   **Defining the role of CNS CD8 T cells in demyelinating disease pathogenisis**

Anne Eliza P. Pugmire;
Brian Evavold (Pathology);
and Hiran Thyagarajan
(Pathology)

Faculty Mentor: Brian Evavold (Pathology, University of Utah)

Overview:

Multiple Sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS), affecting ~2.8 million people worldwide (Walton et al). It is characterized by numbness, paralysis and fatigue due to the

body's immune system attacking its own CNS, with three times higher disease incidence in women. CD4 T cells have been shown to drive the inflammation against the myelin sheath in the CNS; however, other cell types including CD8 T cells are key contributors to MS pathophysiology. The experimental autoimmune encephalomyelitis (EAE) mouse model for MS involves the use of Myelin Oligodendrocyte Glycoprotein (MOG) 35-55 peptide alongside an adjuvant to monitor for disease symptoms including progressive paralysis of the hindlimbs and forelimbs of mice at defined time points. The stages include disease onset (d12-15), followed by peak paralysis (d20-25), which then progresses to a chronic disease stage (d35-100). Our data has identified distinct CD8 T cell subsets which contribute differently to disease kinetics. We observed that the differentiation of CD8 T cell subsets based on CD8α and CD8β protein expression shows a hint of variation in disease progression. The subsets have differential expression of gene targets as confirmed by RNA seq analysis and protein expression by flow cytometry. Specifically, the CD8αα cells show a regulatory expression for Ly49, central memory phenotype and downregulation of exhaustion markers.

Significance:

While much research has been done on conventional CD8 T cells in the CNS which express an alpha-beta heterodimer in the context of MS and EAE, there has been little investigation into the role of non-conventional CD8 cells that express an alpha homodimer (CD8αα cells). These cells could play a crucial role in disease pathogenesis, as they have been identified to have a regulatory phenotype in other tissues (Konkel et al, 2011).

Hypothesis:

While EAE is classically defined as a CD4 T cell mediated

response, the importance of CD8 T cells in promoting demyelination cannot be overruled. Mice with the CD8 receptor gene knockout exhibit reduced disease severity. Moreover, CD8 T cells have been shown to have sustained inflammation in the presence of CD4 T cells. Thus, we hypothesize:

- CD8αα and CD8αβ subsets expand differentially and have unique contributions throughout the course of EAE progression

- CD8αα take on a regulatory phenotype
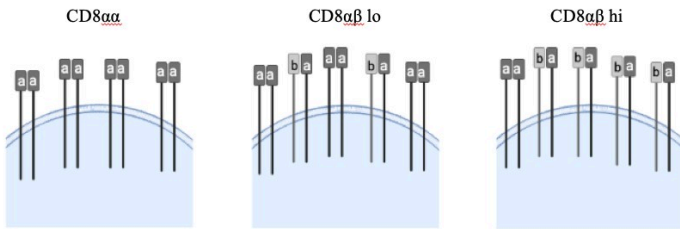
Figure 1: Each CD8 T cell has a variety of CD8 coreceptors, some of which are composed of one α and one β chain while others have two α chains. CD8αβ is the conventional CD8 T cell coreceptor that is thymus selected. However, we have also observed that there are varying levels of CD8 αα cells, which are exclusively composed of the CD8α coreceptor. Varied or mixed expression of αα and αβ coreceptors could regulate demyelinating disease outcome.

Results:

To better understand the role of CD8αα cells in the EAE model, we chose to focus on two timepoints, peak and late chronic. Here, we examined a correlation between disease severity with distribution, function and gene expression of different CD8 T cell subsets during demyelinating disease progression. While the total number of CD8 T cells remained

the same across all timepoints and severity (Fig 2A), we observed a significant increase in bulk CD8 percentages for mice at late chronic stage than for mice at peak EAE (Fig 2B). Total lymphocyte cell count was increased in late chronic mice, although the percentage of total T cells was not significantly different between the two stages (Fig 2C and 2D).
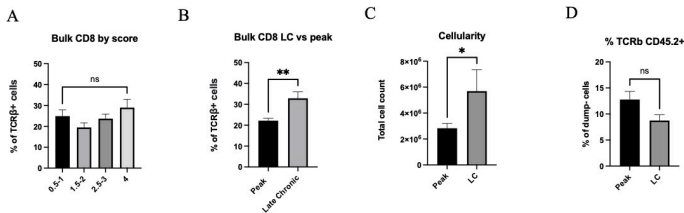
Figure 2: A) Bulk CD8 percentages across varying scores in CNS tissue. B) Bulk CD8 percentages compared between peak and late chronic timepoints. C) Total cellularity for peak *vs* late chronic (LC) mice. D) Percentage of TCRβ+ CD45.2+ cells for peak *vs* late chronic mice. Means + SEM from >3 experiments, with n=11 mice at peak and n=7 mice at late-chronic stage. ns= not significant, *p<0.1,**p<0.01.

Furthermore, through flow cytometric analysis of CNS CD8 T cells, we observed three subpopulations, CD8αα, CD8αβ low, and CD8αβ high. Mice without EAE induction The percentages of the CD8αα subset is higher in sick EAE mice than those where there was no disease induction (Fig 3A), indicating a functional role in demyelination. Additionally, when compared between the spleen and the CNS, of both sick and control mice, the CD8αα percentages in the spleen were negligible (data not shown). In contrast to bulk CD8 cells, CD8αα subset percentages correlated with score but not with the stage of disease (Fig 3B&C). At the late chronic timepoint, percentages were separated and analyzed based on severity, and we observed a significant increase in CD8αα cells in mice with a

low score (Fig 3D). This increase in CD8αα cells in mice that have recovered to a lower disease severity score after a long disease course suggests a potential regulatory role of these cells in EAE disease progression.
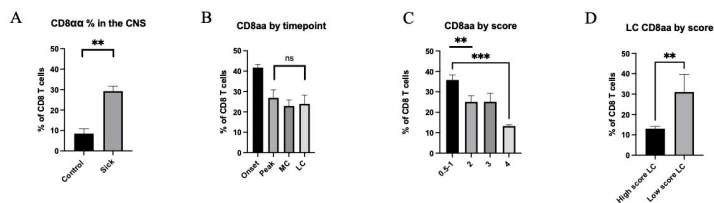
Figure 3: A) Percentage of CD8αα cells compared between non sick and sick EAE mice compiled from all timepoints and with various scores. B) Percentage of CD8αα across timepoints. C) Percentage of CD8αα across scores. D) Percentage of CD8αα in LC compared between low scores (1) and high scores (3-4). Means + SEM from >3 experiments, with n=5 mice at onset, n=10 at peak, n=3 at mid chronic and n= 10 mice at late-chronic stage. ns= not significant, *p<0.1, **p<0.01, ***p<.001

We also examined the expression pattern of various key proteins across the three different CD8 subsets. These candidates were chosen based on results from single cell RNA sequencing data that were obtained from mice at peak and chronic EAE and from studies that had reported increased expression in CD8αα IELs. Expression of KLRK (NKG2D), Ly6C, CD122, NK1.1 and Ly49C was significantly greater in CD8αα cells than in the two CD8αβ expressing subsets (Fig 4A). Expression of CD44 and CD62L was also highest in the CD8αα population, suggesting that this subset takes on a central memory phenotype (Fig 4B).
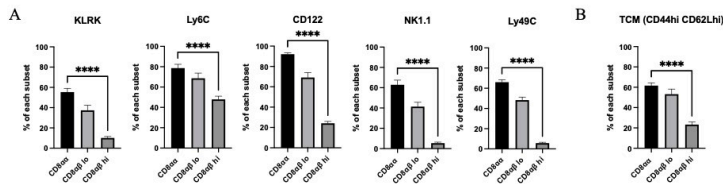
Figure 4: A) KLRK, Ly6C, CD122, NK1.1 and Ly49C expression for the three CD8 T cell subsets were analyzed from mice at peak and late-chronic EAE stages of varied scores. B) T central memory markers were analyzed from mice at peak and late-chronic EAE stages of varied scores. Means + SEM from >3 experiments, with n=10 mice. ****$p<0.0001$

Conclusion:

While we attempt to understand the role and function of the CD8$\alpha\alpha$ cells in CNS during demyelination, our preliminary data suggests a correlation between this cell population with disease severity. Further investigation is necessary to elucidate the source of this CD8$\alpha\alpha$ population (i.e. is it a downregulation of CD8β receptor on traditional CD8 T cells that gives rise to this population or do they migrate in from other areas of the body following disease induction?). In order to do this, we have ongoing experiments measuring the affinity of the TCR of CD8$\alpha\alpha$ cells for peptide MHC to see how it compares to traditional CD8$\alpha\beta$ cells. We also plan to look at the effect of FTY720, a treatment for MS that sequesters mature lymphocytes into secondary lymphoid tissues and prevents them from migrating to the CNS. An analysis of the potential change in CD8$\alpha\alpha$ abundance following this treatment will allow us to better understand the source of this cell population.

References

Crayton, Heidi J, Howard S. Rossman, Managing the symptoms of multiple sclerosis: A multimodal approach,

Clinical Therapeutics, Volume 28, Issue 4, 2006, Pages 445-460, ISSN 0149-2918, https://doi.org/10.1016/j.clinthera.2006.04.005.

Hauser, S. L., Bhan, A. K., Gilles, F., Kemp, M., Kerr, C., & Weiner, H. L. (1986). Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Annals of Neurology*, *19*(6), 578–587. https://doi.org/10.1002/ana.410190610

Konkel, J. E., Maruyama, T., Carpenter, A. C., Xiong, Y., Zamarron, B. F., Hall, B. E., Kulkarni, A. B., Zhang, P., Bosselut, R., & Chen, W. J. (2011). Control of the development of CD8αα+ intestinal intraepithelial lymphocytes by TGF-β. *Nature Immunology*, *12*(4), 312–319. https://doi.org/10.1038/ni.1997

Salehi, Z., Doosti, R., Beheshti, M., Janzamin, E., Sahraian, M. A., & Izad, M. (2016). Differential frequency of CD8+ T cell subsets in multiple sclerosis patients with various clinical patterns. *PLOS ONE*, *11*(7). https://doi.org/10.1371/journal.pone.0159565

Sheng, H., Marrero, I., Maricic, I., Fanchiang, S. S., Zhang, S., Sant'Angelo, D. B., & Kumar, V. (2019). Distinct plzf+cd8αα+ unconventional T cells enriched in liver use a cytotoxic mechanism to limit autoimmunity. *The Journal of Immunology*, *203*(8), 2150–2162. https://doi.org/10.4049/jimmunol.1900832

Walton, Clare et al. "Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition." *Multiple sclerosis (Houndmills, Basingstoke, England)* vol. 26,14 (2020): 1816-1821. doi:10.1177/1352458520970841

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74. The Development of Astrocytes in Zebrafish and its Impact on Vanishing White Matter

Sarv Raafati

Faculty Mentor: Joshua Bonkowsky (Pediatrics, University of Utah)

A leukodystrophy is identified as a class of rare genetic diseases that attack the brain, spinal cord, and peripheral nerves, focusing on either the abnormal development or destruction of white matter, i.e., the myelin sheath. This destruction of the sheath leads to the dramatic decline in

function of the nerves. Most patients with the disease present symptoms in infancy or early childhood, and can develop severe neurological deficits. One of the most common types of leukodystrophies is Vanishing White Matter disease (VWM), which has no treatment other then supportive care. The Bonkowsky lab has modeled VWM using zebrafish to try and study potential gene therapy treatments. Previous research in the field done by Dooves et al. though linked astrocytes of the central nervous system to VWM showing impaired growth of astrocytes in VWM was linked to disease progression. The aim of this project was to genetically prevent the expression of eIF2B5 within astrocytes and see how they were impaired in their in vivodevelopment as a result. To create this model CRISPER Cas9 Vector was created with a Glast+ promoter in order to target and study the effect of the disease on the astrocytes. This vector was injected into wildtype zebrafish. These fish were then in crossed for two generations. The second generation (F2) was then subjected to a 10-day survival curve before completing an HRMA analysis of their DNA to see if any mutation was present. The survival curve done on the F2 generation fish had only 18 fish out of an original 851 over 10 days die. Comparing the survival curve of the F2 fish to that of that performed previously with Keefe et al's work, if the mutation of eIF2B5 had been present a larger die off would be expected. This fact is further compounded and proven with the lack of editing shown in the HRMA results from these F2 fish. This indicates that the genetic mutation was not transgenetic and is not a desirable path to continue forward with for a genetic therapy target. Though this line of fish proved to be unfruitful in the attempt to make a successful animal model, future research though can build off the base laid out in this project and serve as a jumping off point for future studies.

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75. **Research Reflection by Sarv Raafati**

Sarv Raafati

Faculty Mentor: Joshua Bonkowsky (Pediatrics, University of Utah)

My undergraduate research experience has been the highlight of my undergraduate degree. It's been highly motivating, informative, and shaping to my goals and career path. Having Dr. Bonkowsky as a mentor was amazing and he helped me strive for my career goals and aims. The lab environment was incredible. I feel extremely lucky to have participated as much as I did in research at the university.

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76. **Investigating the Role of Microglia Function in the Brain**

Emily Rhodes

Faculty Mentor; Naveen Nagarajan (Human Genetics, University of Utah)

Background

The purpose of this study is to investigate the physiological function in the microglia in response to behavior induction in mice. Grooming is a normal behavior among most animal species. The gene Hoxb8 is required for normal grooming behavior in mice. A mutation in this gene results in pathological over-grooming behaviors in mice that can be related to OCD behaviors in humans. Hox genes are primarily involved in body patterning. Microglia functions as the brain's monitor and responder of immune activity. It was then discovered in 2010 by Capecchi and Chen that the cell in the

brain that implements grooming behavior are microglia cells derived from Hoxb8. The findings of this research shed light on how the brain generates complex behaviors such as OCD. Such mechanistic insight is necessary to understand other neuropsychiatric and neurodegenerative diseases such as Autism, Dementia, and Alzheimer's disease.

Methods

The mice in this study are composed of a specific genetic cross between Hoxb8-IRESCre (male) and GCaMP5 (female). The results of this cross will produce a new generation of offspring that will exhibit GCaMP5 expressed in Hoxb8-positive cells. There are two methods being used to induce specific behaviors in the mice: the first method is a micro water sprinkle assay to induce grooming behaviors and the second method is a visual looming assay to induce anxiety behaviors. Microglia uses calcium to communicate with neurons to drive behaviors. To measure the fluctuations in calcium levels during the behavioral shifts, a calcium sensor called GCaMP will be used. A miniature lens will be inserted into the brain and attached to a miniaturized integrated microscope that will be used to inspect the calcium fluctuation changes mediated by the GCAMP sensor. Using this procedure, there will be 5 minutes of prestimulation, 10 minutes of during behavioral stimulation, and 10 minutes of post-stimulation recording.

Results

This is an ongoing study, however, it is expected that microglia will produce calcium transients in response to the behavioral shifts in the mice. The nature of calcium transients is dependent on the intensity of the behavior; if there is more grooming happening, there is an expectation of having more calcium transients.

Conclusions

Genetic lineage tracing identified that the Hoxb8 gene is turned on only in microglia (approximately 30%) in the brain. In conducting the proposed study, we anticipate measuring calcium transients in microglia during behavioral shifts. However, hypothetically, there can be an unexpected limitation to this experiment in that anxiety behavior can shut the calcium transients down.

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77. **My Lung Health: Shared Decision Making For Lung Cancer Screening**

Grace Richards

Faculty Mentor: Victoria Lynn Tiase (Biomedical Informatics, School of Medicine, University of Utah)

ABSTRACT

Lung cancer is the deadliest cancer in the United States. In 2019, around 140,000 deaths occurred in the Untied States due to lung cancer. Low-dose CT screening to detect lung cancer is an effective tool for preventing these deaths, but the screening has some harms associated with it in addition to the benefits. It has been established that patients have better outcomes when information about lung cancer and low-dose CT lung cancer screening is readily available to them. This

allows them to participate in effective shared decision-making (SDM) regarding the decision to be screened for lung cancer. To facilitate effective SDM, SDM tools must be accessible to patients as well as clinicians. Despite this knowledge, a comprehensive patient-facing tool for lung cancer screening shared decision making is not currently available in many settings. We intend to inform the design of a doctor-prescribed, patient-facing mobile health tool to provide patients with information about their risk for lung cancer and the lung cancer screening process. We explore the user needs, perceived usefulness, and perceived ease-of-use of a mobile-based shared decision-making tool for patients. Our methodology included focus groups and surveys to solicit patient's needs and assess their perception of usefulness and ease-of-use. Overall, the participants' feedback supports the further development of a tool. We found many valuable recommendations to improve the usefulness and ease-of use of a patient-facing SDM tool.

The recommendations will be used to inform the next steps in the development process. Including patient perspectives (user needs) in the design of a web-based tool has the potential to empower patients in the participation of SDM and lung cancer screening, leading to better health outcomes and decreasing mortality from lung cancer in the U.S.

INTRODUCTION

Lung cancer screening is one of the most effective cancer screenings in the United States due to its potential to save 10,000 lives per year; however, it is also one of the most underutilized, with only around 5% of eligible patients being screened [1]-[2]. This is especially true in minority and underserved populations [3]. One way to increase lung cancer screening rates among eligible patients is to implement

informatics tools that guide shared decision-making (SDM) between patients and providers [4]-[5].

Shared decision making (SDM) is defined as: "…a process in which both the patient and healthcare professional work together to decide the best care plan for the patient," [6]. This process makes a special effort to consider the patient's values, goals, and preferences in contrast to traditional clinical decision-making. The use of SDM in clinical practice occurs when both the patient and the provider are informed about the risks and benefits of the treatment for the patient personally and all the potential options for care regarding the medical treatment in question [7]-[8]. SDM is considered the standard when it comes to clinical practice regarding decision making about lung cancer screening and is encouraged by many national and international organizations including the US Preventative Services Task Force which recommends that LCS should not occur without a SDM process [5], [9]-[12]. In fact, the Centers for Medicare and Medicaid Services (CMS) requires that SDM used with patient decision aids for eligible patients be covered since 2015 [9]-[10]. The reason why SDM is particularly useful in lung cancer screening decisions is that the variability in the risks and benefits from one patient to the next depending on age, years of smoking, exposure to carcinogens, and other patient measures [9], [13]-[14]. Thus, a simple "yes/no" approach to lung cancer screening does not sufficiently account for patient preference, and a more nuanced, risk-benefit analysis is required for true shared decision-making. While it has been shown that clinicians, too, prefer a SDM process in patient decision making, especially in lung cancer screening [15], in actual clinic visits they often do not reach this standard of care [9], [16]-[19]. Based on an expert panel from the American College of Chest Physicians

(CHEST), low-dose CT screening for lung cancer is beneficial procedure but has a "tenuous balance of benefit and harm," that is patient sensitive [14], [20]. Therefore, decision aids that include risk prediction calculators are becoming increasingly preferred in lung cancer screening guidelines [12], [21].

One example of an SDM tool is *ScreenLC* (Center for Health Communications Research, University of Michigan, Ann Arbor, MI), an electronic web-based decision aid utilized by providers at the University of Utah Health. This application guides a provider in understanding a patient's risk profile and includes an individualized risk calculator (Fig. 1). The tool is meant to be reviewed by the provider with patients during the patient visits. However, the clinician's knowledge of the risks and benefits of the procedure is not enough. To have an effective SDM process, patients must also be empowered to understand and make decisions together with their providers. Many patients, unfortunately, do not have access to information about their risks and benefits in advance of an appointment with their provider [51]. Anecdotally, providers have requested that patients access the information provided by *ScreenLC* in the form of a prescribed informatics tool before meeting to discuss lung cancer screening.

Our team is investigating the design and development of a new mobile-based tool that patients would have access to at any time after it is prescribed by their provider, both prior to a visit and after they see their provider. The tool could benefit from elements such as information adapted to a health illiterate population, effective and succinct delivery of personalized information, and most importantly, encourage questions for the SDM process between the patient and a provider [17], [18], [52]. The user requirements, usefulness and ease-of-use of such

a tool is still uncertain, necessitating continued research of the topic.
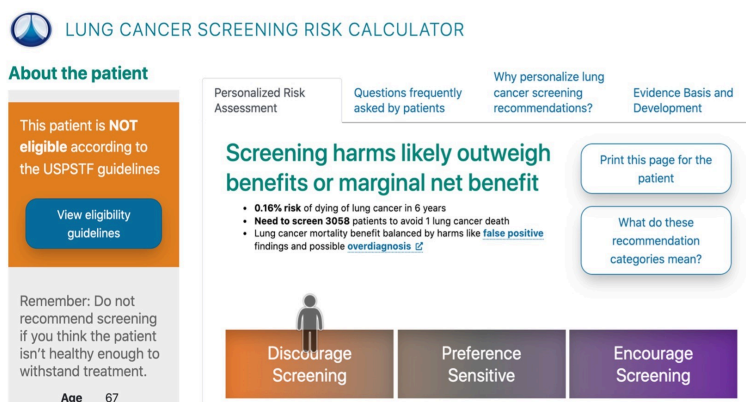
Figure 1. A screening recommendation from the ScreenLC tool for providers with example patient demographics from ScreenLC.com

The goal of the overarching project is to empower patients to make informed decisions about their lung health. The larger study aims to build a web and mobile-based application to aid patients in understanding the risks and benefits of lung cancer screening and update their smoking history within their medical records. The new mobile-based tool will be based on the *ScreenLC* application. It will be modified from *ScreenLC* to a patient-facing application accessible at any time to prepare for SCM discussion about lung cancer screening. We want to ensure the application is culturally appropriate for at-risk populations, specifically African Americans and Hispanics.

The research outlined in this thesis describes user feedback related to the usefulness and ease-of-use of the current clinician focused tool as well as the perceived usefulness and ease-of-use of a patient-facing version. We conducted focus groups and surveys to collect qualitative and statistical

quantitative data regarding patient's views of ScreenLC as if it were to be a patient-facing version of this application. The results will be used to provide recommendations for the design of a patient facing SDM tool. In turn, we anticipate that this research will support the design of SDM tools that empower a broad population of patients to make informed decisions about lung cancer screening. Ultimately, the resulting SDM may improve the lung health of our population by increasing the screening rate of eligible patients leading to fewer deaths nationwide from Lung Cancer.

BACKGROUND

Lung cancer screening is an underutilized preventative measure with the potential to save many lives. Despite the US Preventive Services Task Force recommending low-dose CT screening for eligible patients, adoption is low [2], [22]. They recommend screening for anyone who currently smokes or has quit within the past 15 years, has a more than 30-year smoking history, and is between 55-80 years of age [5], [22]. These guidelines were based on the National Lung Screening Trial (NLST). The NLST was a seven-year trial launched in 2002, where 53,454 patients were randomly assigned to low-dose CT screening or chest radiography to determine the effectiveness of CT screening. The low-dose CT had a 20% reduction in mortality compared to the chest radiography group [5], [23]. Based on this study, it is estimated that 10,000 lives could be saved each year if eligible patients in the top 60th percentile for risk of lung cancer were screened. This trial and the recommendations from the U.S. Preventive Services Task Force establish the credibility and guidelines for screening. Unfortunately, less than 5% of eligible people currently undergo screening for lung cancer [1], [2]. Lung cancer is the deadliest cancer, accounting for 25% of cancer deaths [1],

[24]. Despite this, the screening rate of eligible patients for lung cancer is much lower than the screening rates for other cancers, with the screening rates in 2019 being 76.4% for breast cancer, 37.0% for prostate cancer, and 68.8% for colorectal cancer [24]-[25]. The reason for this is multifactorial, but one barrier to screening is eligible patients lacking accurate information [21].

Despite the benefits of screening, deciding to be screened for lung cancer is not a simple choice for even eligible patients. Low-dose CT screening has potential risks. There is a high false-positive rate in CT screening, resulting in patients receiving unnecessary and possibly harmful procedures [13], [26]. There can be long-term health effects from radiation exposure [27]-[28]. Additionally, patients need to be screened annually which is a long-term commitment to care, and a financial burden that many patients are not willing or able to commit to [29]. Whether or not a patient should be screened for lung cancer varies greatly depending on the patient. This decision is dependent on a patient's smoking history, exposure to secondhand smoke and asbestos, and other demographic circumstances. Because of the varied risk, SDM between a provider and a patient is strongly suggested when deciding whether a patient should be screened for lung cancer. Accordingly, CMS requires the use of SDM for at-risk patients to be covered for screening [11]-[12].

A decision tool to facilitate SDM by providing patients with evidence-based, personalized information about options, risks, benefits, and costs of a medical treatment is recommended [8]. The purpose of SDM tools is to ensure that patients are supported, well-informed and have ownership over their healthcare. *ScreenLC* is an example of an electronic decision aid that provides this kind of information to clinicians to

encourage informed discussions with patients regarding screening for lung cancer. It is integrated into the University of Utah's electronic health record. However, one key aspect of SDM: giving the patient access to this information directly and prior to their visit to access at any time, is still missing [21].

Patients must have access to personalized and evidence-based information about lung cancer screening to be fully engaged in the SDM process [16]. In preliminary discussions with clinicians who currently use *ScreenLC*, there is a desire to have patients access the same information that the clinicians are privy to in order to ensure that their patients are well informed before and after their visit. The intent is to reduce the stress of difficult decision-making by preparing the patient with information ahead of the visit, like whether to be screened for lung cancer.

There is little known regarding the user needs for such a tool. However, it has been established that comprehensive information to meet the high information needs of the target user population is important for a SDM tool [17], [18], [21]. In general, patients want more information regarding screening decisions. In addition, an interactive tool that uses plain language has been shown to be favorable [17]. A few lung cancer screening tools have shown high levels of general acceptability, but the user requirements are lacking [17], [18], [52].

Underserved and minority populations have low screening rates and higher mortality from lung cancer [3]. Additionally, as more healthcare interventions are moved online, there is a growing concern about the inequities in access to these healthcare tools between majority and minority groups [30]. We aspire to address this increasing divide by making the mobile-based patient facing application easily accessible and

adapted to minority populations. As part of this goal, we have ensured these groups are represented in our study and incorporate their feedback into the development of our tool.

METHODS

To build an SDM tool in a clinical setting, first, it was necessary to gather user needs and assess the perceived usefulness (PU) and perceived ease of use (PEU) of the tool. Perceived usefulness measures to what extent potential users of technology perceive that the technology would enhance their experience in a particular domain, in this case the domain is their lung health [31]. Perceived ease of use measures the degree to which a potential user perceives that using a particular tool would be free from effort [31]. Our procedures included a literature review, consulting with domain experts, and soliciting input from target users through a focus group and a survey. Focus group participants were shown the *ScreenLC* application with a demonstration of the personalized risk calculator. The potential for adaption to a patient-facing tool was described. Participants were asked for verbal feedback through focus groups and quantitative feedback with a post-focus group survey.

A. Literature Review

For our literature review, hundreds of papers related to cultural adaption of informatics tools, SDM and SDM decision tools were reviewed. The process allowed us to survey the current literature, note any gaps in the standard of care, and establish a framework for our focus group and survey. We extracted the author, year, title, abstract, principal/relevant points, and a rating for relevance for each paper. Our first inclusion criteria was that the topic of the paper must be cultural adaption, shared decision making informatics tools, or shared decision making. Our second inclusion criteria was that

the paper must be published in the past 10 years. We excluded papers that were not related to informatics tools.

B. Focus Group Questions, Script

Our purpose statement for our focus group and post focus group survey was to better define the general user needs and the PU and PEU of a web-based tool to inform the further design and 10

development of the patient-facing tool.

To plan and finalize details of the focus group the team consulted experts, including community engagement resources at the University of Utah. The inclusion and exclusion criteria, the recruitment process, the number of participants and number of focus groups, and the duration of the focus group were finalized. A REDCap (Vanderbilt University, Nashville, TN) survey (Appendix: Table 1A) was developed for collection of demographic data and screening participants.

The inclusion criteria were developed based off the USPSTF screening guidelines, which are the guidelines recommended by the American Cancer Society (ACS) [12]. The exclusion criteria were developed based on the constraints of the focus group. Potential participants were excluded if they did not self-identify as 'very comfortable' or 'somewhat comfortable' with video conferencing (Table I). They were also excluded if they did not complete the screening survey including all of the demographic information (Appendix: Table A1).

Our goal for each group was to include at least two individuals who self-identified as Black and at least two individuals who self-identified as Hispanic.

An original draft of the focus group questions that would serve as a basis for the facilitator's script during the focus group were created using a framework similar to the 'Sample Focus Group Moderator's Guide' in to *Making Health*

Communication Programs Work [32]. Specifically, the section '*Steps in Developing and Pretesting Messages and Materials*' (pg. 185) was used to guide our development of the focus group process. The first draft consisted of 28 questions. Through various iterations and expert consensus, we finalized seven questions for the focus group (Table II).

TABLE I: CRITERIA FOR PARTICIPATION IN FOCUS GROUPS

INCLUSION CRITERIA	EXCLUSION CRITERIA
• Age 50 to 80 years old • 20+ pack years • Current smoker or has quit within the past 15 years	• Comfortable using video conference • Past or current Lung Cancer diagnosis • Incomplete screening survey

TABLE II: FOCUS GROUP QUESTIONS

A. What comes to mind when you think about lung cancer and lung cancer screening?
B. What are your thoughts about talking with your doctor about lung cancer screening?
C. What information in the application would you want to see before visit with your provider? How about after a visit with your provider?
D. How would this tool be useful to you as a patient?
E. What might be some barriers for you or individuals like yourself to using this tool?
F. What suggestions do you have to make the application easier to use/understand?
    a. What should be added for patients?
    b. What should be removed for patients?
G. What do you think of the risk calculator?

C. Post-Focus Group Survey Development

We began by selecting six measures related to the perceived usefulness (PU) and perceived ease of use (PEU) for the post-focus group survey. These measures were informed by the Technology Acceptance Model (TAM) questionnaire as a general framework [31], [34]. The questions were modified further using '*Constructing Questionnaires Based on The Theory of Planned Behavior*' [33] and close collaboration with Dr. Kimberly Kaphingst (University of Utah), an expert in health communications. Based on the Theory of Planned Behavior, the behavior in question for our study was a participant's use of the tool. There were several drafts that were reviewed by three members of the team including Dr. Kaphingst before

finalization. We included six questions total with three of the questions aimed at measuring perceived usefulness (PU) of the tool and three questions aimed at measuring perceived ease of use (PEU) of the tool (Table III).

TABLE III: SURVEY QUESTIONS

Perceived Usefulness Measures	Behavior Measurement
This tool contains information that I want to know before making a decision about whether to be screened for lung cancer.	DPB
I would use a tool like this if it was recommended by my provider.	IP
With the help of this tool, I would feel confident that I would be able to discuss cancer screening with my provider.	DBC

Perceived Ease of Use Measures	Behavior Measurement
I will be able to access this tool.	DAB
It will be convenient for me to use this tool.	DPB
The information in this tool is easy to understand.	IPB

DAB: Direct measurement of actual behavioral control
DPB: Direct measurement of perceived behavioral control
IPB: Indirect measurement of perceived behavior control
IP: Intention performance

For each question, respondents would rate their 'percent agreement' on a scale from 0 to 100 with 100 being full agreement, 50 being neutral, and 0 being full disagreement (Fig. 2).
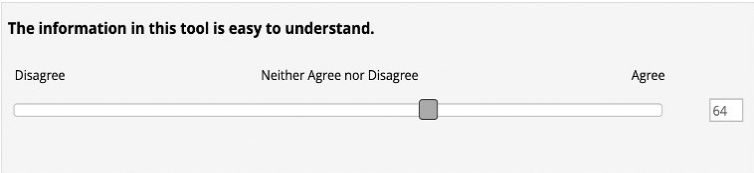
Figure 2. Display that respondents saw while filling out the post-focus group survey with a scale that goes from 0 to 100 with 100 being full agreement.

C. Focus Group Procedures

The methods for focus group recruitment included direct outreach through collaboration with the Huntsman Cancer Institute's (Salt Lake City, UT) Health Outcomes and Population Equity (HOPE) program for tobacco cessation, and the University of Utah's Community Collaboration &

Engagement Team. We also distributed flyers to Facebook (Menlo Park, Ca) pages, in person at University of Utah clinics, and ResearchMatch (Vanderbilt University, Nashville, TN). We contacted individuals who met our inclusion criteria in the HOPE program database through email to be a part of our focus group. Each person received a QR code linked to the REDCap screening survey (Appendix).

The focus groups met for a recorded video conference session over Zoom (Zoom Video Communications, San Jose, CA) that lasted approximately 2 hours in duration. The focus groups took place in the second half of 2022 and early 2023.

After introductory remarks, consent review, and discussion about questions A-B (Table II) participants were given a short presentation of the tool and provided some background information about lung cancer and low-dose CT screening (Fig. 3). They were also provided with an example demonstration of how a patient would use the risk-calculator (Fig. 3). The facilitators then asked the participants the focus group questions C-G (Table II). At the completion of the focus group, participants were asked to complete post-focus group survey. All participants were compensated for their time.

About the patient

Is this patient healthy enough for screening? ⓘ

Yes   No

Age

Sex   Male   Female

Race or Ethnicity   ○ Non-Hispanic White

○ Non-Hispanic Black/African American

○ Hispanic

○ Other

Years Smoked

Has quit smoking?   Yes   No

Packs   Cigarettes

Average packs per day

COPD or Emphysema?   Yes   No
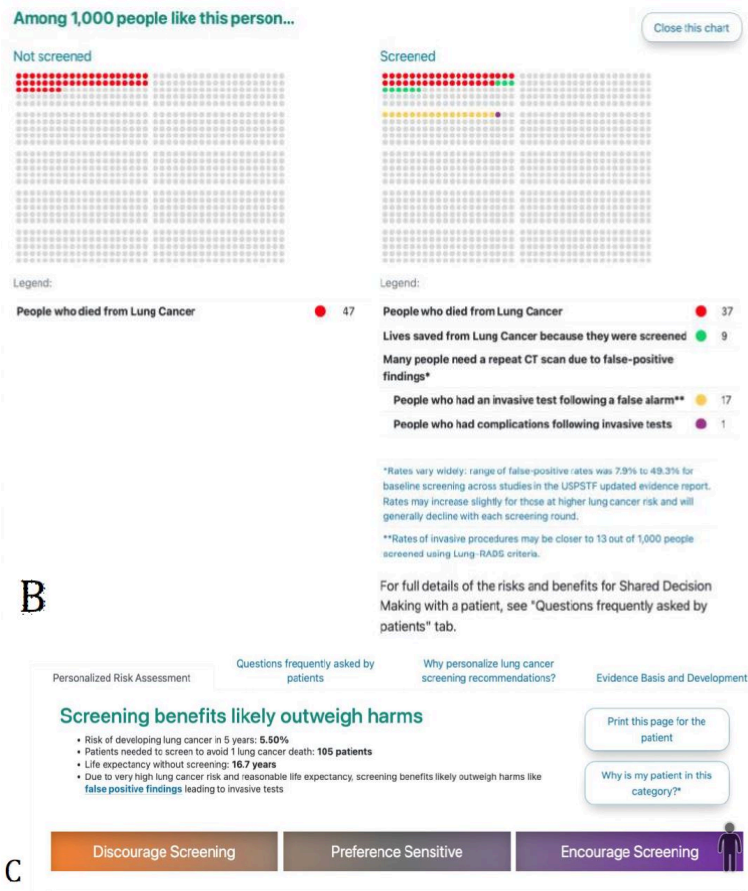
Figure 3. ScreenLC Display. [A] The data entry user interface screen, this is the first page that a user of ScreenLC sees **[B] A** visual representation of the patient's risk profile using a dot probability display **[C]** The personalized screening recommendation for the Patient

Upon completion of all four focus groups, zoom audio recordings were downloaded and securely transmitted to a transcription service. The deidentified transcripts were uploaded into Dedoose (dedosse.com, Inc.). The team conducted a thematic analysis by coding participant responses through

consensus and then identified themes and patterns. From the themes and 15

patterns, recommendations were formulated for the design and development of a patient facing SDM tool.

1. *D. Statistical Analyses*

The results from the post-survey were analyzed using descriptive statistics. These results were reported with the mean score and standard deviation for each question. In addition, for each measure the number of respondents that agreed, neither disagreed or agreed, and disagreed with the statement were reported. Participants who responded with a score higher than 60% were considered to be in agreement with the measure, 60-40% was considered neutral, and less than 40% was considered disagreement.

IV. RESULTS

A. Literature Review

We included 19 papers in the literature review [8]–[10], [19], [35]-[48]. Many papers are concerned with the cultural adaption of informatics tools due our original intention to adapt ScreenLC to be more culturally appropriate for Hispanic populations and translate the tool into Spanish [35]-[36], [38]-[44], [46]-[47]. However, through our literature review and anecdotal feedback from clinicians, we determined that there was a greater need to adapt the tool to be patient-facing first. Another major takeaway from the literature review was that SDM does improve patient outcomes but adoption and facilitation of SDM varied widely [8]-[10], [19], [45]. Many papers found that patient empowerment through information and education was key to successful SDM [9], [37], [45].

B. Focus Group Participant Demographics

Through our recruitment efforts, we recruited 23 participants for four focus groups (Table IV). Our first focus group consisted of eight participants, all with documented histories of smoking, including six men and two women. This focus group included three minority participants with one individual who self-identified as African American and two individuals who self-identified as Hispanic. Our second focus group had seven participants, all with documented histories of smoking, including one nonbinary individual and six women. There were two individuals who self-identified as African American in the second focus group. Two women that met our criteria participated in our third focus group. Both participants identified as Caucasian. We had six participants in our fourth focus group including four women and two men. This focus group included two minority participants who both self-identified as Hispanic.

TABLE IV: PARTICIPANT DEMOGRAPHICS

Measure	Percentage of total participants (number)
Age (Mean ± STD)	58 ± 6
Age between 50-60 (%)	61 (14)
Age between 60-70 (%)	35 (8)
Age between 70-80 (%)	4 (1)
Female (%)	61 (14)
Male (%)	39 (9)
Nonbinary (%)	4 (1)
Caucasian (%)	78 (18)
Hispanic (%)	13 (3)
African American (%)	13 (3)
Annual household income (mean ± STD)	45,000 ± 23,000
Annual household income above 45k (%)	48 (11)
Annual household income below 45K (%)	52 (12)

C. Findings from Focus Group Analysis

The responses to our focus group questions were coded using *Dedoose* software for aggregation and analysis. The general findings including the user needs, the facilitators, and barriers to tool use of the perceived tool were extracted from this analysis (Table V).

TABLE V: GENERAL FINDINGS FROM THE FOCUS GROUP

Code: *Main Takeaway*	Participant Quotes

Tool Suggestions – Information/Education: *Overall many participants would like to know more about lung cancer screening*	1. "Oh, I'd want to be able to see, me personally, all of the information but also anything else that could expand it as they look at maybe-- I have no idea all the things that cause lung cancer, but maybe oxygen content, blood pressure, weight. Things like that in conjunction with this tool could also bring about a better understanding of this actual disease."
	2. "But why? What is it that turns the table to help somebody survive. And what is it about the CT scanning that causes so many repeats? When there are a lot of repeats there, that's actually something that I would be very hesitant in in seeing that because even low doses of radiation, it's radiation, which is why I don't like mammograms and when you have to go into the doctor - of course, you have to do this anyway - the dentist and they look at your teeth."
Tool Feedback – Shared Decision Making: *Participants perceive that tool would facilitate shared decision making*	1. "I think it's a great tool for helping a doctor and a patient decide whether or not to have screening, to have that risk versus benefit conversation. And that puts it in, what is for me, an easy-to-understand format. So, I think it'd be very helpful."
	2. "I think anything that you can make your doctor aware of or be informed about between you and your doctor before you actually see them in the office is a wonderful thing, because the doctors are so rushed these days. They don't sit down and spend 25 or 30 minutes with you. They spend about 10."
Tool feedback- Applications/Functions: *More comprehensive factors for data input in the screening recommendation*	1. "My question is this screening for lung cancer only for specifically smoking tobacco products? Or does it include any kind of substances you may have smoked all throughout your years? What is the distinction between what you can get cancer from? I mean, smoking is smoking. Does it really matter if it's a cigarette or if it's a vape or weed or whatever the case may be?"
	2. "As a smoker, I don't know, my experience is that depending where I am in my life dictates how much I smoke. So depending on where you're at, your stress level is, and what is happening in my life, my personal life will definitely dictate-- there were times were I was maybe smoking a pack and a half of cigarettes a day. I'm not doing that anymore because I'm not at that stress level. So yeah, so I can be able to give the accurate information so I can get accurate results"
Tool feedback- Understanding: *Tool should use clear language accessible to all health literacy levels*	1. "Trust me, that's Doctor talk. You need to put it in patient terms."
	2. "Is this being read by a doctor or by the patient? If it's by the patient, it needs to be spoken in lay terms."
Lung cancer screening access: *More cost transparency information in the tool*	1. "I would find it- it would be harder- does that mean that tool is going to set the pay or not to pay with insurance companies? You know what I mean? Because your doctor could say, "Yeah, let's do it. Let's go get your screen." And then the insurance company could say, "Sure, go ahead and get your screening. But you're going to have to pay for it. It's

		not covered by your deductible, so guess what? You're going to pay for the whole thing out of pocket," or whatever."
	2.	"My question is how much is the lung screening going to cost? How much does it cost? I mean, for me, I'm in a study - which I haven't done it this year; I need to see if I can still do my screening - and it doesn't cost me anything. But with the cost of healthcare going up, if you don't have any insurance or you don't have good insurance-- just a thought."
Tool feedback- Probability: *Visuals displaying probability are not clear to participants*	1.	(Regarding the personalized probability display) "So it's kind of generic. It's general. It's not specific enough for me."
	2.	"It's hard to discern on the dots. And then with all those dots, one person's going to be saved. Why do it at all? It doesn't make any sense."
Tool feedback – Privacy: *Participants are concerned about privacy*	1.	"Is this covered by HIPPA? So if you put this information into this questionnaire, then it can't go any further than who's specified on on the form? Or is it? HIPPA doesn't cover this."
	2.	"I think for me a big a big issue would be privacy. If I enter information in there about exactly how much tobacco I've consumed before. Where can it go. And there's some, there's places where I don't want it to go. For instance, if it gets out to say, an insurance carrier, I don't want them knowing what my history of smoking is. Because that might affect my insurability in the future, or even life insurability that sort of thing. And if I didn't believe that my my information was saved, I'd be tempted to lie."

The focus group coding identified user needs relevant to developing our proposed interface. Feedback commonly given related to shared decision making was that the tool would facilitate shared decision making between the participants and their providers. In addition, participants did not always understand clearly the that the tool was meant to be used in the context of shared decision making in a provider visit. The two common misconceptions that participants had related to SDM was that the tool would make a screening decision for them, or the tool was meant to be used by a patient alone and wouldn't be followed by a visit with their provider.

Focus group participants want to be better educated about lung cancer screening. Participants want to see the tool include more general information about lung cancer screening, a common takeaway from the excepts coded into 'Tool

Suggestions – Information/Education'. Some specific information that participants want to see includes a glossary of terms related to screening, information about how the timing of diagnosis influences lung cancer survival rate, and a basic overview the low-dose CT screening lung cancer diagnostic process.

One common takeaway was that the tool would be more useful if the recommendation for screening was more comprehensive and included factors such as other substances used by the patient aside from tobacco, allowed for periods of non-smoking in between smoking, and took into account family history. Excerpts related to this idea were coded into 'Tool feedback- Applications/Functions'. Participants also suggested that they would like to be able to modify the input data to reflect theoretical scenarios and see how that might change their risk for getting lung cancer. In addition, in our 'Tool feedback- Understanding' code, participants expressed a need for the language in the tool to be clear and accessible to all health literacy levels to make the perceived tool easy to use. Some terms that were not understood by participants included 'pack-years', 'screening' in the context of the tool, 'quintile', and 'eligibility' in the context of LCT screening. Potential users perceived that they would want information related to insurance coverage and cost of LCT screening on the tool. Many participants did not understand the relationship between the recommendation given in the tool and their access to insurance coverage for lung cancer screening (Table V: quote 1 and 2, 'Lung cancer screening access').

The probability conveyed on the tool in the dot display (Fig. 3) was overwhelmingly perceived as unclear and confusing to participants and commonly evoked feelings of fear regarding lung cancer ('Tool feedback – Probability'). Participants

frequently did not understand that the display was personalized to their risk for lung cancer (Table V: quote 1, 'Tool feedback – Probability'). Lastly, many participants expressed concerns about their privacy related to their electronic health data as coded in 'Tool feedback – Privacy'. Participants were especially concerned about how or if this information would be accessible to insurers or employers. The most common suggestions coded in these excepts was to make it clear who has access to the data entered on the tool and what measures were taken to protect it (Table V: quote 2, 'Tool feedback – Privacy' quote 2).

TABLE VI: BARRIERS AND FACILITATORS IDENTIFIED IN THE FOCUS GROUP

Code	Barrier/Facilitator	Participant Quote
Barriers to low-dose CT screening	A desire for ignorance	"Not wanting to know, I guess, like Participant said. She'd just as soon as not know. I don't know that I'd necessarily want to know either."
	Hopelessness	"Or they might think it's hopeless because even though if they have these risks, so few people actually are, at this point, cured. And so it takes a lot to go through for a minuscule chance of getting cured. And is that something that is helping to get a larger base of individuals that potentially could be helped? How do you present that in a manner that is more encouraging? Yeah. That's all I'll say."
	Patient/provider relationship	"I think it's kind of interesting, though, that I had to learn about it from my doctor saying, "Hey, you should get the screening since you've quit smoking," but I've had no follow-up... And that was a couple of years ago."
Barriers to tool use	Lack of technology access	"I mean, I think there's a lot of barriers depending on, a lot of people, where they live, whether they have access to the technology. So I mean, cities, it's usually not a problem because there's all kinds of technology, and people have smartphones and computers, but people who live in maybe rural areas where internet might be spotty might have problems trying to access different tools."
	Lack of technology comfort	"For me, it's not a problem, but for some other people in a certain age group who are not used to-- don't have a smartphone or don't have a laptop or a computer, for those people, it would be difficult, I think, to access it and use it to help themselves"
	Fear of lung cancer/shame	"I still agree in the presentation that it may be good to be discouraging in many ways because people can want or perceive they want things that they really don't want or need. But you also don't want to scare those away that really do want or need it or have them feel depressed or hopeless, because that's a huge thing that doesn't help with healing"
Facilitators of tool use	Provider contact	"Hopefully, they have that kind of relationship with their doctors and that's something that can be used as a screening

		tool for their yearly visit maybe, for their yearly physical, or for follow-up visits, that type of thing."
	Technology comfort	"I would want to interact with it. I think I'm pretty good technologically, so if I saw something there on my portal, I would definitely go into it to check it out, just because I'm always all up in the portal, in my chart, in my messages, in my medications and stuff like that."
	Patient empowerment	"But I looked through the screening tool that you had, actually took the screening tool that you had, and it seems like a really-- rather than shaming people to quit smoking, you give them data. I'm a data person. Give me facts. Don't give me scare tactics. I like the facts. And so that interests me. That made me interested enough to want to sit here at 8 o'clock at night on a computer that I don't understand."

Barriers to low-dose CT screening for lung cancer were identified during the focus group (Table VI). Some participants expressed that they did not want to know if they had lung cancer or not; because they were not willing to change their behaviors regardless of the result (Table VI: 'Barriers to low-dose CT screening – A desire for ignorance'). Others shared that they feel powerless to do anything about their risk for lung cancer and had no hope of improving their lung health (Table VI: 'Barriers to low-dose CT screening – Hopelessness'). Feedback given related to this motif was to include in the tool more encouraging messaging on the tool and emphasizing how screening can help a patient take control of their own lung health. One participant said, "Some sort of ending on an encouraging note, because obviously, if somebody is looking at that, doing the tool, they may have indicators that they think that they could be eligible for lung cancer because they smoked ... If you're taking care of your health, you need to be reinforced with that, with the tools." Another added that, "I think there needs to be some layer of hope in there too, that the earlier detection can make a difference." Participants identified

that in some situations their patient to provider relationship was a barrier to getting screened (Table VI: 'Barriers to low-dose CT 24

screening – Patient/provider relationship'). Some reasons given for that were that their provider was not proactive about screening (Table VI: quote, 'Barriers to low-dose CT screening – Negative patient/provider relationship'), they did not feel comfortable bringing it up with their provider or they are not honest with their provider.

Barriers to tool use were also extracted from the focus group (Table VI). This included a lack of access to appropriate technology use for various reasons including disability, physical barriers (i.e. rural or underserved populations) and language barriers (Table VI: 'Barriers tool use– Lack of technology access'). Another barrier discussed is lack of technology comfort or computer illiteracy. The mean age of participants is 58 and 60% of our participants were between 50 and 60 which means that our participants are younger on average than the tool's target population (50 to 80). So, the technology comfort barrier is likely even more prevalent among our general target user population than our results reflect. Lastly, participants identified fear and shame as a reason why they or others like them would not want to have a SDM process with their provider. In potentially related excerpts from the focus groups, stigma around smoking was a common topic and some participants even shared that they felt like, "second-class citizens" because they were smokers and that "smoking has become hush-hush. You don't tell people [that you are a smoker]."

Facilitators for tool use were also coded in our analysis and topics including contact with a provider, technology comfort, and patient empowerment were all associated with perceived

usefulness and ease of use of the tool (Table VI). Having regular contact with a primary care provider was perceived as a facilitator to tool use. Participants who felt empowered as a patient and wanted to be better informed often mentioned that they would use the tool, in contrast to participants who did not want to be better informed about their lung health or felt powerless to change their own lung health.

D. Post-Focus Group Survey Data

All 23 focus group participants answered all questions. Overall, the survey data showed that a patient-facing version of *ScreenLC* is perceived to be useful and relatively easy to use (Table VII, Fig. 4 & Fig. 5).

TABLE VII: MEAN CRITERIA SCORES (N=23)

Measure	Mean Percentage Agreement ± STD [0-100]	Agree 60-100	Neither 40-60	Disagree 0-40
Perceived Usefulness Measures				
1. This tool contains information that I want to know before making a decision about whether to be screened for lung cancer.	80 ± 13	19/23 (83%)	2/23 (8%)	2/23 (8%)
2. I would use a tool like this if it was recommended by my provider.	86 ± 11	19/23 (83%)	3/23 (13%)	1/23 (4%)
3. With the help of this tool, I would feel confident that I would be able to have a discussion about lung cancer screening with my provider	81 ± 10	19/23 (83%)	3/23 (13%)	1/23 (4%)
Perceived Ease of Use Measures				
4. I will be able to access this tool.	86 ± 10	20/23 (87%)	2/23 (8%)	1/23 (4%)
5. It will be convenient for me to use this tool.	85 ± 11	20/23 (87%)	2/23 (8%)	1/23 (4%)
6. The information in the tool is easy to understand	79% ± 10	18/23 (78%)	3/23 (13%)	1/23 (4%)

For each measure of perceived usefulness and perceived ease of use, no more than two participants disagreed with the measure (Table VII). For each question, the mean percentage agreement is over 80% aside from measure 6, with an agreement of 79%. This shows that the participants generally strongly agreed with the statements used to measure PU and PEU. The number of participants who 'agree', 'neither agree nor disagree' and 'disagree' with each measure is listed in Table VII. For each measure, no more than two participants disagreed with each statement.

The overall score for the application's usefulness as perceived by the participants was 82 ± 3 percent, which shows that the application is perceived to be useful (Fig. 4). The overall score for the ease of use of the tool as perceived by the participants was 83 ± 4 percent which shows that the application is perceived to be easy to use (Fig. 5).
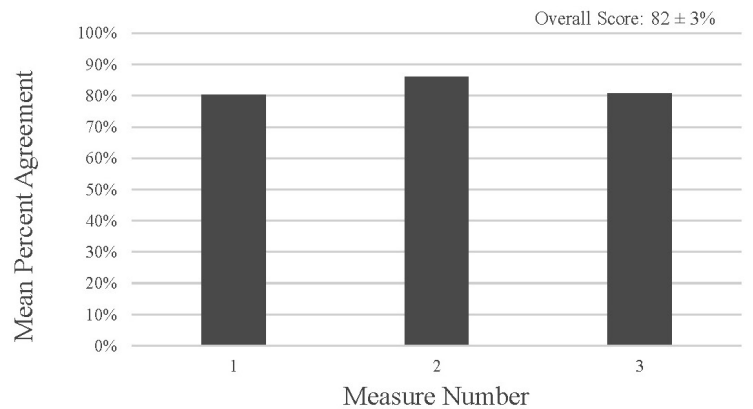
Figure 4. Perceived Usefulness Measures from the survey data have a mean percent agreement of 82 ± 3% indicating that overall participants perceived the tool as *useful*
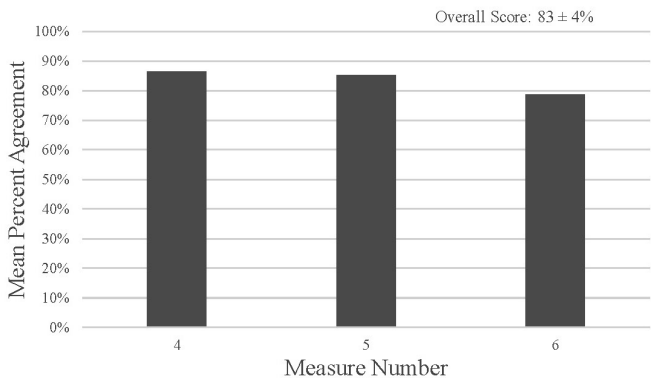
Figure 5. Perceived Ease of Use Measures from the survey data have a mean percent agreement of $83 \pm 4\%$ indicating that overall participants perceived the tool as *easy to use*.

V. DISCUSSION

Lung cancer should not take as many lives as it does in the United States, given the availability of modern, highly accurate lung cancer screening technology. However, this technology is not used by all the patients who could benefit from it. This disconnect between the number of patients who are screened and those who should be screened could be primarily due to a lack of patient knowledge [3]. To better inform patients about the need for screening, a patient-facing SDM tool needs to be developed. A few studies show that decision aids increase patient knowledge about lung cancer and a participant's willingness to be screened when eligible [14]-[16]. However, the literature has not established the desired user experience of a web-based decision aid. Through a needs assessment for perceived usefulness and ease of use, we found a number of recommendations that should be incorporated into future tools.

Low-income populations in the United States are the least health literate of all income groups [49]. As shown in Table IV, our focus group participants had a mean household income of

$45,000 which is considered low-income in the United States and approximately half of our participants are in the low-income bracket [35]. This will allow us to incorporate feedback from the population for which this tool is most needed. While the representation of minority groups in our focus groups was not as large as we wanted, we did have both African American and Hispanic participants. The mean age of our participants was 58 which may mean that our focus group participants are more health literate than our average target user (Table IV) [49].

Our focus group identified that participants wanted clearer visuals, low-dose CT screening education, and insight into how their data would be used (Table V). Many participants requested clarity on how the prediction calculator's data are used and how the prediction was calculated and wanted a more comprehensive screening recommendation. Additionally, participants petitioned for more advice concerning the outcomes of their screening options, i.e., they wanted to know how the timing of the intervention would impact their health. We identified that participants preferred this information to be presented in clear visuals. The most effective presentation mode was determined to be simple graphs and scales. Addition thematic analysis is being conducted for further analysis.

From our analysis, stigma around smoking could be related to the shame and fear that prevents patients from engaging in shared decision making (Table VI). In past research, this has been identified as a barrier to successful lung cancer screening [37]. Depending on the patient and provider relationship, contact with the provider can serve as a facilitator or a barrier for engaging in shared decision making. Additional research is required to understand this relationship and solutions to overcoming this barrier.

We determined that our proposed lung-cancer screening SDM tool is useful and easy to use from the post-focus group survey (Fig. 4 & Fig. 5). This suggests that prospective users will most likely be willing and able to use the tool if it is provided. In the post-focus group survey, participants scored each measure to the extent to which they agree with the measure (Table VII). Participants scored how much they agreed with the statement, 'This tool contains information that I want to know before I make decision about lung cancer with my provider', as a measure of how useful the tool was perceived to be. The average score was 80 ± 13% indicating general agreement with that statement and the largest standard deviation out of all the measures by 2% (Table VII). This result is consistent with the focus group analysis in which participants had a wide variance in how useful they perceived the tool to be. Given feedback from the focus groups about how to include more patient specific information, we would expect this score to increase for the actual tool in which the results of this research would be incorporated. The statement 'I would use a tool like this if it was recommended by my provider' got an average score of 86 ± 11% (Table VII). The next measure of usability scored was 'With the help of this tool, I would feel confident that I would be able to have a discussion about lung cancer screening with my provider' got an average score of 81 ± 10% (Table VII). This is third lowest score out of all the measures. This score could be related to the stigma identified as a barrier to screening in our research as well as others and further research is necessary to understand this stigma and how to overcome it [37]. Our first measure of the tool's perceived ease of use is how respondents scored the statement, "I will be able to access this tool." The average score was 86 ± 11% (Table VII). Next, the statement, "It will be convenient for

me to use this tool," was scored similarly with an average score of 85 ± 11% (Table VII). Lastly, the statement, "the information in this tool is easy to understand," had the lowest score with an average score of 79 ± 10% (Table VII). This is consistent with the focus group and would be expected to increase following incorporation of the focus group feedback. Our quantitative results indicate that continued development of a patient-facing tool would prove an effective use of resources and time to work towards the goal of empowering patients to make informed decisions regarding their lung health. Adaptation from *ScreenLC* will decrease the recourses and time required for the development of the tool.

From the results, we suggest the following recommendations for the next version of the tool, adapted from *ScreenLC,* for patient access at all times:

1. Providers engaging with patients prior to using tool

2. Clear language and messaging on how to use the tool in a shared decision-making process

3. Additional education materials including:

a. How the screening recommendation is determined

b. Basic information about what lung cancer screening is and the diagnostic process

4. Simple visuals and statistics to covey the personalized lung cancer risk

Our results and these recommendations informed by our results are in line with what little has been established regarding the user needs for a lung cancer screening SDM tool. Specifically, the need for more information and clear language [17], [18], [52].

The privacy concerns could be addressed with a SMART (SMART Health IT, Boston Children's Hospital, Boston, MA) authorization flow meaning that all of the data for the tool

would be accessed from the user's device and no backend server would have access to the token that authorizes use of the EHR. The user would be made aware of this through a simple information blurb next to the data entry stating that their information is protected and who can access it.

One strength of our potential tool is that through this analysis, the voice of patients will be taken into account during development. When creating a shared decision-making tool, too often, patients are not included in the process of determining the user requirements and specifications for the tool being developed. In most cases, this lack of insight would decrease the usefulness and ease of use of the tool by patients [39]. Development and testing with patient input is recommended for effective SDM tools [39], [50]. Currently, in lung cancer screening SDM, educational tools or materials have not been utilized effectively and there is a need for highly useable and easy to use tools [48], [51]. In addition, it has been shown that barriers to lung cancer screening should be taken into account when developing decision tools for screening [37]. Many of our findings were not anticipated by our team and will shape our design in significant ways to improve the usability of the end product.

SDM tools in the context of lung cancer screening are shown to be effective in empowering patients when developed to be acceptable and feasible to patients [17], [52]. When patients are empowered with personalized information, screening rates are expected to increase but the extent of this increase is unknown and not agreed upon [9], [15], [53]. Further research is needed regarding the impact of SDM tools on screening rates.

The major limitation of the presented work was the small sample size. With a sample size of 23 we do not have the

statistical power for the study to be representative of the whole target

population but rather just a sample and further research should be done to generalize these results. Additionally, our study aimed to examine the user needs of a specific population of patients. However, informational needs were only gathered from human subjects that could be contacted by our team, which may have excluded target users that our team could not contact. In addition, only English-speaking participants were included in the focus groups. Lastly, there may be a gap between the perceived usefulness and perceived ease of use versus the actual usefulness and ease of use, or the usefulness and ease of use of the developed application.

In conclusion, our study supports the case for the development of shared decision-making tools to assist patients in making potentially life-saving low-dose CT lung cancer screening decisions as patients are interested and perceive the SDM tool to be useful. The methods of this study ensure that patient feedback is incorporated into the final SDM tool. Based on feedback from patients, some recommendations include data sharing should be disclosed, simple visuals or percentages should be used to display probability, and the context of the tool in the SDM process should be explicit on the tool. Much work still needs to be done to verify and validate such tools and establish a dissemination strategy to ensure it reaches the patients who need it the most. The intention is that through the development of web-based, mobile-accessible SDM tools, we should be able to increase the utilization of low-dose CT screening by eligible patients and ultimately decrease lung cancer mortality.

VI. APPENDIX

TABLE A1
RECRUITMENT SURVEY

First name:

Last name:

Email:
Phone number:
Preferred method of contact:
  a.   Email
  b.   Phone call
  c.   Text

How did you hear about this opportunity? May select multiple.
  a.   Past participant email
  b.   Flyer
  c.   Community Collaboration & Engagement Team Facebook Page
  d.   Social media posts (e.g. Facebook, Instagram, etc.)
  e.   Friend
  f.   Word-of-mouth
  g.   Other
Are you a current smoker?
  a.   Yes
  b.   No
How many years have you smoked?
In the years that you smoked, on average how many packs per day did you smoke?
  a.   9 or fewer cigarettes
  b.   ½ pack
  c.   ¾ pack
  d.   1 pack
  e.   1 ½ pack
  f.   2 packs
  g.   2 ½ pack
  h.   Other
Have you ever had a CT scan (CAT scan) for lung cancers?
  a.   Yes
  b.   No
Have you ever been diagnosed with lung cancer?
  a.   Yes
  b.   No
  c.   Unsure
When was the last time you had an annual check-up or well-visit with your doctor?
  a.   This year
  b.   1-2 years ago
  c.   2-5 years ago
  d.   More than 5 years ago
Age:
State:
Zip code:
Gender identity:
  a.   Man
  b.   Woman
  c.   Transgender
  d.   Non-binary
  e.   Other
Race(s)
  a.   African American

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    b.  Alaskan Native
    c.  Asian
    d.  Black
    e.  Caucasian
    f.  Hispanic/Latino
    g.  Native American
    h.  Native Hawaiian / Pacific Islander
    i.  Other
Total annual household income
    a.  Less than $10,000
    b.  $10,000-$24,999
    c.  $25,000-$39,999
    d.  $40,000-$49,999
    e.  $50,000-$74,999
    f.  $75,000 or more
Highest level of education
    a.  High school
    b.  Some college
    c.  Associate degree
    d.  Bachelor's Degree
    e.  Master's Degree
    f.  Doctorate Degree
    g.  Other
How would you describe the area where you live?
    a.  Rural (population not included within an suburban or urban area; typically have a population of
        less than 2,500)
    b.  Suburban (population of at least 2,500 and less than 50,000)
    c.  Urban (population of 50,000 or more)
Technology Access:
Do you feel comfortable using video conference (e.g. Zoom)?
    a.  Very comfortable
    b.  Somewhat comfortable
    c.  Slightly comfortable
    d.  Not at all comfortable
If selected to participate, which of the following gift cards would you prefer?
    a.  Amazon
    b.  Target
If selected to participate, how would you like your gift card delivered?
    a.  Email
    b.  USPS mail
Can we share your contact information with the research team so they can invite you to participate in a paid
individual interview, via zoom, to advise on the design of a lung-cancer screening benefits calculator?
    a.  Yes
    b.  No
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VII. ACKNOWLEDGEMENTS

VIII. REFERENCES

[1] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2020," *CA Cancer J Clin*, vol. 70, no. 1, pp. 7–30, Jan. 2020, doi: 10.3322/caac.21590.

[2] A. L. Association, "New Report: Critically Low Lung Cancer Screening Rates Reveal Opportunity to Save More

Lives." https://www.lung.org/media/press-releases/state-of-lung-cancer-2022 (accessed Jan. 11, 2023).

[3] E. Sosa *et al.*, "Racial and Socioeconomic Disparities in Lung Cancer Screening in the US: A Systematic Review," *CA Cancer J Clin*, vol. 71, no. 4, pp. 299–314, Jul. 2021, doi: 10.3322/caac.21671.

[4] A. C. Melzer, S. E. Golden, S. S. Ono, S. Datta, K. Crothers, and C. G. Slatore, "What Exactly Is Shared Decision-Making? A Qualitative Study of Shared Decision-Making in Lung Cancer Screening," *J Gen Intern Med*, vol. 35, no. 2, pp. 546–553, Feb. 2020, doi: 10.1007/s11606-019-05516-3.

[5] R. Wender *et al.*, "American Cancer Society lung cancer screening guidelines," *CA: A Cancer Journal for Clinicians*, vol. 63, no. 2, pp. 106–117, 2013, doi: 10.3322/caac.21172.

[6] "Definition of shared decision making – NCI Dictionary of Cancer Terms – NCI," Feb. 02, 2011. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/shared-decision-making (accessed May 03, 2023).

[7] Y.-C. T. Shih, Y. Xu, L. M. Lowenstein, and R. J. Volk, "Implementation of Shared Decision Making for Lung Cancer Screening Among the Privately Insured Nonelderly," *MDM Policy & Practice*, vol. 6, no. 1, p. 2381468320984773, Jan. 2021, doi: 10.1177/2381468320984773.

[8] G. Elwyn *et al.*, "Shared Decision Making: A Model for Clinical Practice," *J GEN INTERN MED*, vol. 27, no. 10, pp. 1361–1367, Oct. 2012, doi: 10.1007/s11606-012-2077-6.

[9] A. T. Brenner *et al.*, "Evaluating Shared Decision Making for Lung Cancer Screening," *JAMA Internal Medicine*, vol. 178, no. 10, pp. 1311–1316, Oct. 2018, doi: 10.1001/jamainternmed.2018.3054.

[10] A. M. O'Connor *et al.*, "Toward The 'Tipping Point': Decision Aids And Informed Patient Choice," *Health Affairs*,

vol. 26, no. 3, pp. 716–725, May 2007, doi: 10.1377/hlthaff.26.3.716.

[11] C. B. [D-N.-15 Rep. Rangel, "Text – H.R.3590 – 111th Congress (2009-2010): Patient Protection and Affordable Care Act," Mar. 23, 2010. http://www.congress.gov/ (accessed Mar. 31, 2023).

[12] R. A. Smith *et al.*, "Cancer screening in the United States, 2018: A review of current American Cancer Society guidelines and current issues in cancer screening," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 4, pp. 297–316, 2018, doi: 10.3322/caac.21446.

[13] "Lung Cancer Screening, Version 3.2018, NCCN Clinical Practice Guidelines in Oncology in: Journal of the National Comprehensive Cancer Network Volume 16 Issue 4 (2018)." https://jnccn.org/view/journals/jnccn/16/4/article-p412.xml?rskey=gCbwL3&result=1318&utm_source=TrendMD&utm_medium=cpc&utm_campaign=JNCCN_TrendMD_1 (accessed May 03, 2023).

[14] P. J. Mazzone *et al.*, "Screening for Lung Cancer: CHEST Guideline and Expert Panel Report," *Chest*, vol. 153, no. 4, pp. 954–985, Apr. 2018, doi: 10.1016/j.chest.2018.01.016.

[15] A. C. Melzer, S. E. Golden, S. S. Ono, S. Datta, K. Crothers, and C. G. Slatore, "What Exactly Is Shared Decision-Making? A Qualitative Study of Shared Decision-Making in Lung Cancer Screening," *J Gen Intern Med*, vol. 35, no. 2, pp. 546–553, Feb. 2020, doi: 10.1007/s11606-019-05516-3.

[16] G. Elwyn *et al.*, "Developing a quality criteria framework for patient decision aids: online international Delphi consensus process," *BMJ*, vol. 333, no. 7565, p. 417, Aug. 2006, doi: 10.1136/bmj.38926.629329.AE.

[17] K. K. McDonnell *et al.*, "Developing and testing a brief clinic-based lung cancer screening decision aid for primary

care settings," *Health Expectations*, vol. 21, no. 4, pp. 796–804, 2018, doi: 10.1111/hex.12675.

[18] D. S. Reuland, L. Cubillos, A. T. Brenner, R. P. Harris, B. Minish, and M. P. Pignone, "A pre-post study testing a lung cancer screening decision aid in primary care," *BMC Medical Informatics and Decision Making*, vol. 18, no. 1, p. 5, Jan. 2018, doi: 10.1186/s12911-018-0582-1.

[19] O. Karnieli-Miller and Z. Eisikovits, "Physician as partner or salesman? Shared decision-making in real-time encounters," *Social Science & Medicine*, vol. 69, no. 1, pp. 1–8, Jul. 2009, doi: 10.1016/j.socscimed.2009.04.030.

[20] F. C. Detterbeck, P. J. Mazzone, D. P. Naidich, and P. B. Bach, "Screening for Lung Cancer: Diagnosis and Management of Lung Cancer, 3rd ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines," *Chest*, vol. 143, no. 5, Supplement, pp. e78S-e92S, May 2013, doi: 10.1378/chest.12-2350.

[21] R. J. Volk and D. Stacey, "Ensuring High-Quality Shared Decision-making for Lung Cancer Screening," *JAMA Oncology*, vol. 8, no. 11, pp. 1561–1562, Nov. 2022, doi: 10.1001/jamaoncol.2022.3766.

[22] US Preventive Services Task Force *et al.*, "Screening for Lung Cancer: US Preventive Services Task Force Recommendation Statement," *JAMA*, vol. 325, no. 10, pp. 962–970, Mar. 2021, doi: 10.1001/jama.2021.1117.

[23] "National Lung Screening Trial: Questions and Answers – NCI," Sep. 18, 2002. https://www.cancer.gov/types/lung/research/nlst-qa (accessed Mar. 31, 2023).

[24] M. B. Schabath and M. L. Cote, "Cancer Progress and Priorities: Lung Cancer," *Cancer Epidemiol Biomarkers Prev*, vol. 28, no. 10, pp. 1563–1579, Oct. 2019, doi: 10.1158/1055-9965.EPI-19-0221.

[25] "Cancer Prevention & Early Detection| American Cancer Society." https://www.cancer.org/research/cancer-facts-statistics/cancer-prevention-early-detection.html (accessed Mar. 31, 2023).

[26] E. F. Patz Jr *et al.*, "Overdiagnosis in Low-Dose Computed Tomography Screening for Lung Cancer," *JAMA Internal Medicine*, vol. 174, no. 2, pp. 269–274, Feb. 2014, doi: 10.1001/jamainternmed.2013.12738.

[27] J. M. Albert, "Radiation Risk From CT: Implications for Cancer Screening," *American Journal of Roentgenology*, vol. 201, no. 1, pp. W81–W87, Jul. 2013, doi: 10.2214/AJR.12.9226.

[28] D. A. Pierce and D. L. Preston, "Radiation-Related Cancer Risks at Low Doses among Atomic Bomb Survivors," *Radiation Research*, vol. 154, no. 2, pp. 178–186, Aug. 2000, doi: 10.1667/0033-7587(2000)154[0178:RRCRAL]2.0.CO;2.

[29] P. B. Bach *et al.*, "Benefits and Harms of CT Screening for Lung Cancer: A Systematic Review," *JAMA*, vol. 307, no. 22, pp. 2418–2429, Jun. 2012, doi: 10.1001/jama.2012.5521.

[30] M. Warschauer, Technology and Social Inclusion: Rethinking the Digital Divide. MIT Press, 2004.

[31] F. D. Davis, "Perceived Usefulness, Perceived Ease of Use, and User Acceptance of Information Technology," *MIS Quarterly*, vol. 13, no. 3, pp. 319–340, 1989, doi: 10.2307/249008.

[32] "Making health communication programs work." https://stacks.cdc.gov/view/cdc/24017 (accessed Mar. 31, 2023).

[33] J. Francis *et al.*, "Constructing questionnaires based on the theory of planned behaviour: A manual for Health Services Researchers.," *Quality of life and management of living resources; Centre for Health Services Research*, 2004, Accessed: Mar. 31, 2023. [Online]. Available: https://abdn.pure.elsevier.com/en/publications/constructing-questionnaires-based-on-the-theory-of-planned-behavi

[34] "Comparison of Four TAM Item Formats: Effect of Response Option Labels and Order – JUX," *JUX – The Journal of User Experience*, Aug. 30, 2019. https://uxpajournal.org/tam-formats-effect-response-labels-order/ (accessed Mar. 31, 2023).

[35] M. Barrera, F. G. Castro, L. A. Strycker, and D. J. Toobert, "Cultural adaptations of behavioral health interventions: a progress report," *J Consult Clin Psychol*, vol. 81, no. 2, pp. 196–205, Apr. 2013, doi: 10.1037/a0027085.

[36] A. K. Borondy Kitts, "The Patient Perspective on Lung Cancer Screening and Health Disparities," *J Am Coll Radiol*, vol. 16, no. 4 Pt B, pp. 601–606, Apr. 2019, doi: 10.1016/j.jacr.2018.12.028.

[37] L. Carter-Harris and M. K. Gould, "Multilevel Barriers to the Successful Implementation of Lung Cancer Screening: Why Does It Have to Be So Hard?," *Annals ATS*, vol. 14, no. 8, pp. 1261–1265, Aug. 2017, doi: 10.1513/AnnalsATS.201703-204PS.

[38] F. Cartujano-Barrera *et al.*, "Feasibility and Acceptability of a Culturally- and Linguistically-Adapted Smoking Cessation Text Messaging Intervention for Latino Smokers," *Front Public Health*, vol. 8, p. 269, Jun. 2020, doi: 10.3389/fpubh.2020.00269.

[39] V. Chenel, W. B. Mortenson, M. Guay, J. W. Jutai, and C. Auger, "Cultural adaptation and validation of patient decision aids: a scoping review," *Patient Prefer Adherence*, vol. 12, pp. 321–332, 2018, doi: 10.2147/PPA.S151833.

[40] J. S. Choi *et al.*, "Cultural Adaptation of a Community-Based Hearing Health Intervention for Korean American Older Adults with Hearing Loss," *J Cross Cult Gerontol*, vol. 34, no. 3, pp. 223–243, Sep. 2019, doi: 10.1007/s10823-019-09376-6.

[41] C. Escoffery *et al.*, "A systematic review of adaptations of evidence-based public health interventions globally," *Implementation Science*, vol. 13, no. 1, p. 125, Sep. 2018, doi: 10.1186/s13012-018-0815-9.

[42] C. J. Etzel *et al.*, "Development and validation of a lung cancer risk prediction model for African-Americans," *Cancer Prev Res (Phila)*, vol. 1, no. 4, pp. 255–265, Sep. 2008, doi: 10.1158/1940-6207.CAPR-08-0082.

[43] K. L. Kumpfer, M. Pinyuchon, A. Teixeira de Melo, and H. O. Whiteside, "Cultural adaptation process for international dissemination of the strengthening families program," *Eval Health Prof*, vol. 31, no. 2, pp. 226–239, Jun. 2008, doi: 10.1177/0163278708315926.

[44] F. F. Marsiglia and J. M. Booth, "Cultural Adaptation of Interventions in Real Practice Settings," *Res Soc Work Pract*, vol. 25, no. 4, pp. 423–432, Jul. 2015, doi: 10.1177/1049731514535989.

[45] A. C. Melzer, S. E. Golden, S. S. Ono, S. Datta, K. Crothers, and C. G. Slatore, "What Exactly Is Shared Decision-Making? A Qualitative Study of Shared Decision-Making in Lung Cancer Screening," *J Gen Intern Med*, vol. 35, no. 2, pp. 546–553, Feb. 2020, doi: 10.1007/s11606-019-05516-3.

[46] K. Resnicow, R. Soler, R. L. Braithwaite, J. S. Ahluwalia, and J. Butler, "Cultural sensitivity in substance use prevention," *Journal of Community Psychology*, vol. 28, no. 3, pp. 271–290, 2000, doi: 10.1002/(SICI)1520-6629(200005)28:3<271::AID-JCOP4>3.0.CO;2-I.

[47] R. J. Volk *et al.*, "Feasibility of a patient decision aid about lung cancer screening with low-dose computed tomography," *Preventive Medicine*, vol. 62, pp. 60–63, May 2014, doi: 10.1016/j.ypmed.2014.02.006.

[48] G. X. Wang *et al.*, "Barriers to Lung Cancer Screening Engagement from the Patient and Provider Perspective," *Radiology*, vol. 290, no. 2, pp. 278–287, Feb. 2019, doi: 10.1148/radiol.2018180212.

[49] K. T. Hickey *et al.*, "Low health literacy," *Nurse Pract*,

vol. 43, no. 8, pp. 49–55, Aug. 2018, doi: 10.1097/
01.NPR.0000541468.54290.49.

[50] M. A. Smith, "The Role of Shared Decision Making in
Patient-Centered Care and Orthopaedics," *Orthopaedic Nursing*,
vol. 35, no. 3, pp. 144–149, May 2016, doi: 10.1097/
NOR.0000000000000243.

[51] S. P. E. Nishi *et al.*, "Shared Decision-Making for Lung
Cancer Screening: How Well Are We 'Sharing'?," *Chest*, vol.
160, no. 1, pp. 330–340, Jul. 2021, doi: 10.1016/
j.chest.2021.01.041.

[52] R. J. Volk *et al.*, "Effect of a Patient Decision Aid on Lung
Cancer Screening Decision-Making by Persons Who Smoke:
A Randomized Clinical Trial," *JAMA Network Open*, vol. 3, no.
1, p. e1920362, Jan. 2020, doi: 10.1001/
jamanetworkopen.2019.20362.

[53] M. I. Fukunaga *et al.*, "Tools to Promote Shared
Decision-Making in Lung Cancer Screening Using Low-Dose
CT Scanning: A Systematic Review," *Chest*, vol. 158, no. 6, pp.
2646–2657, Dec. 2020, doi: 10.1016/j.chest.2020.05.610.

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78.   **Understanding the Role of Noncanonical Wnt Signaling Adrenal Biology and Cancer**

Catherine Rousculp

Faculty Mentor: Kaitlin Basham (Oncological Sciences, University of Utah)

The Wnt signaling pathway plays an important role in development and homeostasis andis aberrantly activated in many human cancers. There are two main Wnt pathways: the canonicalWnt pathway, involving regulation of b-catenin; and the noncanonical Wnt pathway, whichmediates signaling through other effector molecules. One tissue where Wnt signaling is criticalfor growth and maintenance is the adrenal gland. In cases of adrenocortical carcinoma (ACC), anaggressive and rare cancer in the adrenal cortex, the Wnt

signaling pathway is aberrantlyactivated. ZNRF3 is a negative regulator of Wnt signaling and loss-of-function mutations inZNRF3 is one of the most frequent genetic alterations in ACC. ZNRF3 promotes the turnover ofthe WNT receptor FZD, which can act together with coreceptors for both the canonical andnoncanonical pathways. Because FZD has the potential act through both pathways, ZNRF3 maybe involved in the regulation of both canonical and noncanonical Wnt signaling. To examine thenoncanonical pathway, we looked at the WNT coreceptor RYK and found that combinedconditional loss of Ryk and Znrf3 in the adrenal reduces the hyperplasia seen in mice withconditional Znrf3 loss alone. We also observed an increase in myeloid immune infiltration in 6-week female mice when both genes were absent. These results suggest that ZNRF3 interacts withRYK, influencing noncanonical Wnt signaling, and that Ryk loss in the absence of Znrf3 mayplay a role in immune infiltration in the adrenal. The finding that ZNRF3 interacts with bothcanonical and noncanonical signaling may have important implications for treatment of ACC andtargeting upstream events in the Wnt pathway.

About the Author

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79. **Study of the Mechanism of Action of Chloroquine And Neuroinflammation in a Zebrafish Model of X-Linked Adrenoleukodystrophy**

Dana Schiwal and Joshua Bonkowsky (Pediatric Neurology)

Faculty Mentor: Joshua Bonkowsky (Pediatrics, University of Utah)

X-linked Adrenoleukodystrophy (X-ALD) is neurodegenerative disease caused by mutations in the ABCD1 gene, which is believed to affect the myelin in the central nervous system. X-ALD is one of the most common leukodystrophies, however, there are no good experimental mice models for the disease. The Bonkowsky lab developed a zebrafish model for X-ALD, as zebrafish are an ideal model due to being relatively inexpensive, quick to mature, and good for high-throughput screening. Using this model chloroquine was identified as a promising drug treatment for X-ALD. However, the zebrafish model the Bonkowsky lab was using lost its decreased motor behavior phenotype in the abcd1 mutant fish likely due to SNPs, which also indicated that the Abcd1 gene began to be expressed in the mutant fish, which was not suitable for the mutant to be a proper model. Therefore, the first aim of this project was to evaluate and characterize several new ABCD1 zebrafish lines. The second aim of this project was to use one or more of these ABCD1 zebrafish lines to characterize the mechanism of action of chloroquine and to characterize its potential to control the inflammatory response. We evaluated three different fish lines (sa21198, sa34298, and a CRISPR knockout line) for a motor behavior phenotype at 5-, 6-, and 7-days post-fertilization as a preliminary way to select a good model. We did not observe a decreased motor behavior phenotype in any of the abcd1 mutant fish compared to wild-type fish for any of the three lines. We then evaluated the RNA expression levels of Abcd1 for wildtype and mutant fish of each line to ensure that even without a motor phenotype, the fish were still viable as a model. At this time work has

not been done yet on characterizing the mechanism of action of chloroquine due to the time-consuming characterizing and figuring out how to genotype these three fish lines. However, going forward the sa21198 and the CRISPR KO line has been selected to work with due to ease of genotyping. The CRISPR KO line is also going to be used in the future for several different experiments.

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SECTION IX

Mines & Earth Sciences

80. Assessing Yellowstone Seismic Velocity Structure Using a Dense Seismic Array

Chloe Barry; Jamie Farrell
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and Fan-Chi Lin (Geology
and Geophysics)

Faculty Mentor: Fan-Chi Lin and Jamie Farrell (Geology &
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The Yellowstone magmatic system represents one of the largest continental silicic volcanic centers, notable for three explosive eruptions occurring in the last 2.1 million years [1,2]. The volcanic system is marked by frequent earthquakes,

episodic ground deformation, high heat flux, and hydrothermal features [1,2]. The system is underlain by two magma reservoirs that are largely constructed of stacked sill complexes [2] and is fed by a west-northwest dipping plume in the upper mantle. The plume generates basaltic partial melts which collect in the lower sill complex located in the lower crust. These partial melts then further fractionate, and the lessdense silicic melt rises to the upper rhyolitic sill complex located in the upper crust [2]. These two sill complexes are likely connected by dikes and have been imaged through seismic tomography, however the resolution of the models is often limited by the earthquake-station ray path distribution. In order to improve these models, it is necessary to improve the ray path coverage and identify areas of the models that do not agree with the new observations.

To achieve this, a temporary dense array of 608 Zland 3-component geophones were deployed along the main roads of Yellowstone National Park from 08/18/2020 through 09/21/2020 (Figure 1).
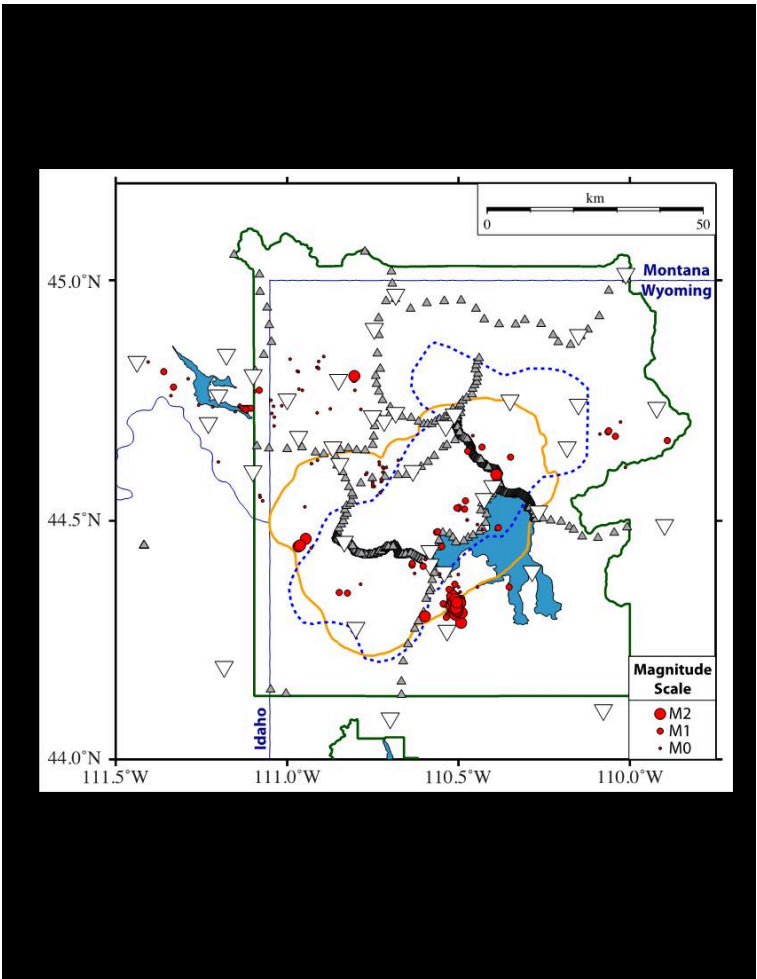
Figure 1: A map of Yellowstone showing nodal stations labeled as grey triangles and permanent stations as white inverted triangles. The orange line outlines the 0.63 Ma Yellowstone caldera, and the blue dashed line represents the spatial extent of the upper-crustal magma reservoir. Red circles represent earthquakes that occurred during the nodal deployment and are sized based on magnitude.

This is the first dense array of its kind to be deployed in

Yellowstone. In this study, we measured body wave arrivals, or P-wave and S-wave arrivals, from local earthquakes across the dense array to better understand the subsurface magmatic structure of Yellowstone. The measured travel times are compared with predictions based on a 1D reference velocity model. The observed travel time discrepancies are then used to assess areas where the model needs to be adjusted (Figure 2).
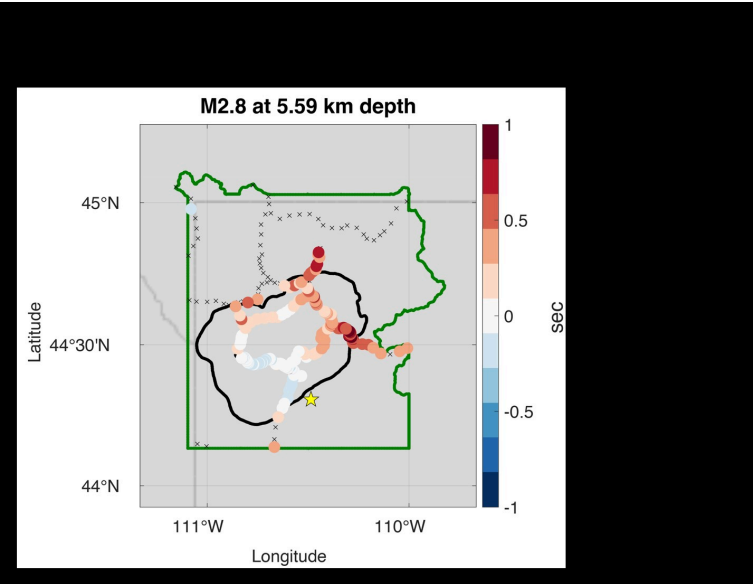
Figure 2: A map of the nodal stations color coded based on the P-wave misfit in seconds for the M 2.8 earthquake. The black line is the 0.63 Ma Yellowstone caldera. Red color coding is for positive misfit where the dense array data velocities are slower than the model, and blue color coding is for negative misfit where the dense array data velocities are faster than the model. The yellow star represents the earthquake location, and the green line represents the boarder of Yellowstone National Park. The black crosses represent stations where no travel time picks were made.

We will use body wave ray tracing to determine the 3D sensitivity of our measurements and to understand how the Yellowstone magma body, which has low velocity, contributes to our observation. This process of modeling and comparing will help us to evaluate the most recent 3D velocity model of Yellowstone [2] and provide insight for a future tomographic inversion.

[1] Farrell, J., R. B. Smith, S. Husen, and T. Diehl (2014), Tomography from 26 years of seismicity revealing that the spatial extent of the Yellowstone crustal magma reservoir extends well beyond the Yellowstone caldera, Geophys. Res. Lett., 41, doi: 10.1002/2014GL059588

[2] Huang, H.H., F.-C. Lin, B. Schmandt, J. Farrell, R.B. Smith, and V.C. Tsai (2015), The Yellowstone magmatic system from the mantle plume to the upper crust, Science, 348, doi: 10.1126/science.aaa5648

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81. Creation of Landslide Database in Iceland Assessing Mechanisms, Occurrences, and Hazards

Claire Cruz and Leif Anderson (Geology and Geophysics)

Faculty Mentor: Leif Anderson (Department of Geology)

Abstract

A landslide is the movement of rock, debris, or earth down a slope. Landslides are serious geological hazards often triggered by tectonics, glaciation, and erosion. This research identifies

the locations and parameters of landslides in Iceland by creating the first comprehensive landslide database of Iceland. Iceland has few trees making it easy to identify the landslides, and the rock type is almost entirely basaltic (with some rhyolite) making it easier to track trends. Iceland was completely covered by ice during the last age and glaciers helped form the steep fjord topography near the coasts. coasts.

Introduction

Iceland is characterized by its tectonic activity and periods of glaciation. These geologic processes have resulted in a diverse range of landscapes, including mountains, glaciers, volcanoes, and fjords. However, these geological features also make Iceland prone to natural hazards such as landslides. Landslides are a significant threat to the infrastructure, economy, and environment of Iceland. Understanding causes and impacts of landslides is crucial for developing effective strategies for hazard mitigation and disaster management.
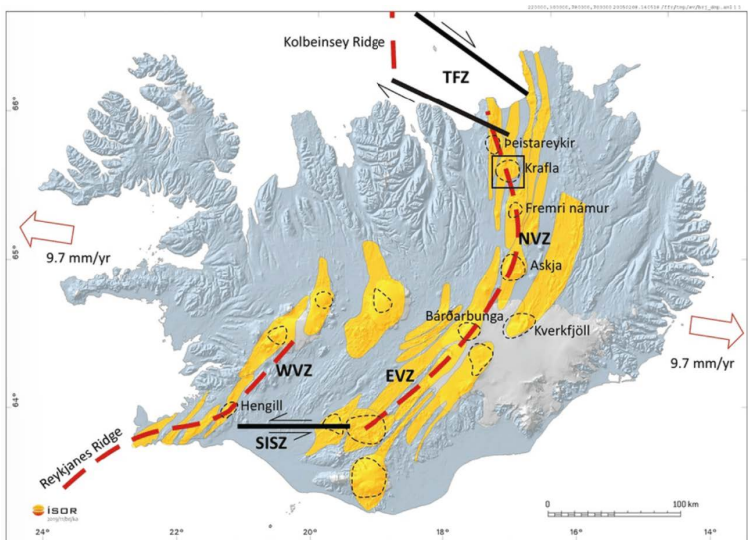
Figure 1: Tectonic map of Iceland. A Mid Ocean Ridge divides

Iceland and is an active spreading center. Yellow-colored regions around the MOR indicate active volcanic regions and central volcanos are outlined in black. Iceland is spreading at a rate of 9.7mm/yr.
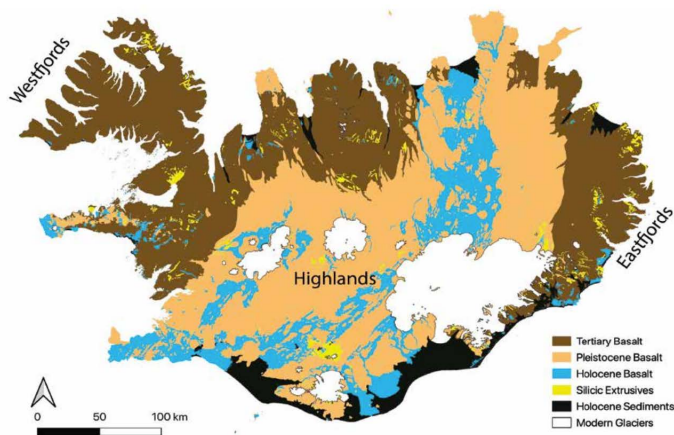
Figure 2: Geologic Map of Iceland. The rock age increases from the spreading center [MOR described in Figure 1]. The youngest topography is modern glaciers and the oldest is tertiary basalt. Silicic intrusions appear to speak sporadically around Iceland and indicate the presence of former central volcanos that are composed of weak montmorillonite or rhyolitic clay.

Methods

We used aerial and satellite imagery from Google Earth and Landmælinga Íslands Geodesy of Iceland. Hummocky tomography and abrupt topography changes often indicate the presence of a landslide. In Google Earth, we have digitized just over 2,000 landslides and run analyses on the collected data using Python and ArcGIS. In addition, we have created a series of plots to present findings.
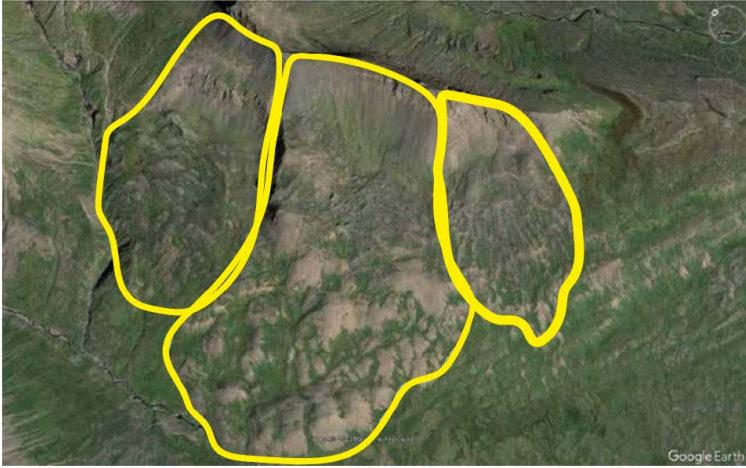
Figure 3: Example of 3 landslides outlined in Google Earth

Results and Discussion

Much of Iceland's topography is composed of fjords which are long, narrow, deep inlets formed by the erosion of valleys and mountains by glaciers. The process of fjord formation began during the last Ice Age, approximately 18,000 years ago. As the glacier moves, it erodes the bedrock beneath it, creating U-shaped valleys. Over time these U-shaped valleys erode and create steep V-shaped valleys that are weak and highly susceptible to landslides. In Iceland, these fjords are concentrated in the northern, eastern, and western regions, and our findings indicate that most of the landslides are concentrated in these areas with some exceptions being landslides around former central volcanos or areas of particularly steep topography. Tectonics also plays a large role in Iceland's landscape. The MOR that runs through Iceland serves as a spreading center and an active site for volcanism. Central volcanos form along the MOR and propagate outward eroding over time and becoming over steepened hillslopes subject to failure. Topography near the MOR is shallow dipping

and dips increase as you move farther from the spreading center.
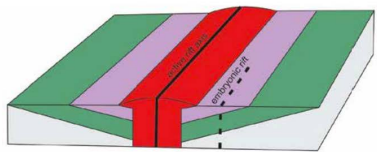
Topography near MOR has shallow dipping layers.

Topography near central volcanos has steeply dipping layers.
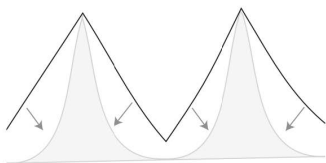
Pre Pleistocene Iceland was not glaciated (mainly composed of river shaped valleys). The last ~2 million years glaciers have covered celand and created "U" shaped valleys with steeper dips.

Figure 4: Different topography and steepness of hillslopes.
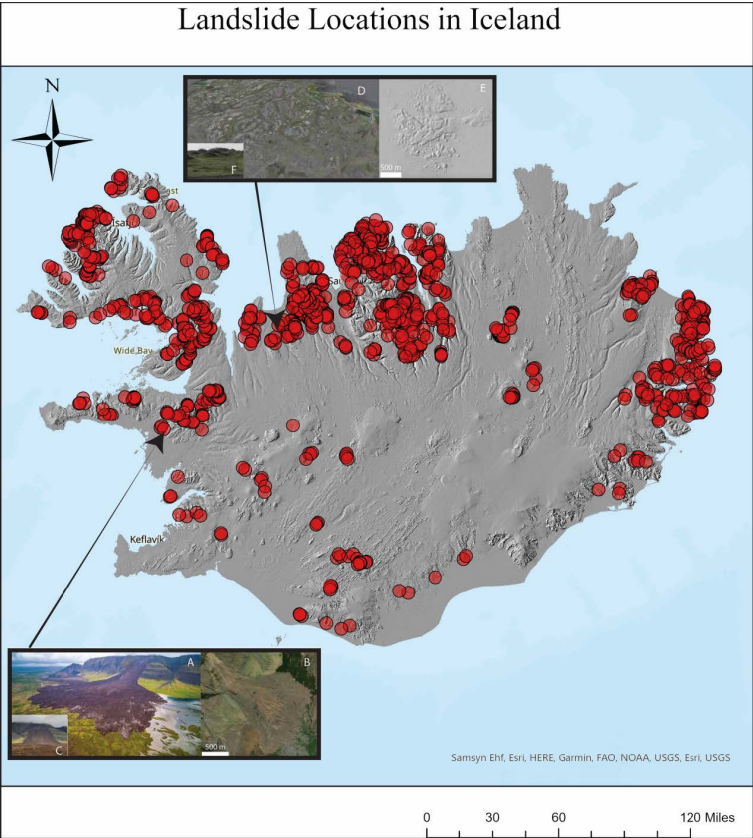
Figure 5: Red dots represent individual landslide locations we have identified. Images A-F are photos of landslides at specific locations.
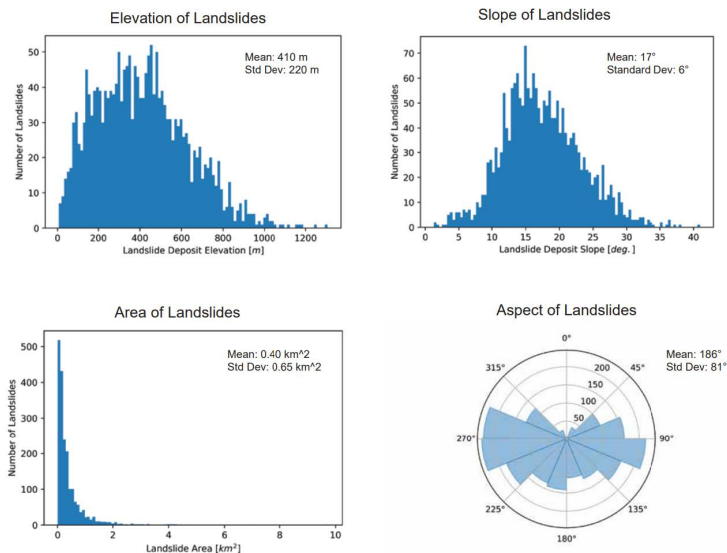
Figure 6: We created plots of Elevation, slope, area, and Aspect. We calculated the mean elevation as 410m with a standard deviation of 220, mean slope as 17 degrees with a standard deviation of 6 degrees, mean area as .04 square Km with a standard deviation of .65 square Km, mean aspect as 186 degrees with a standard deviation of 81 degrees.
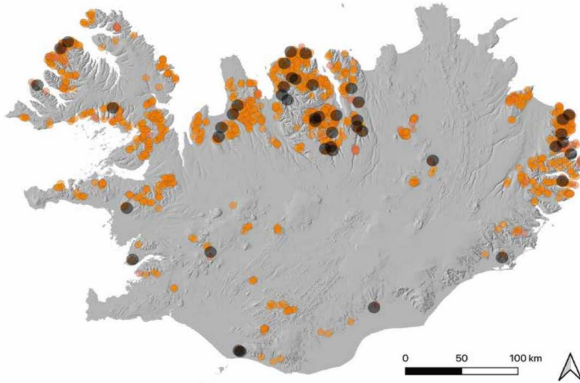
Figure 7: Orange dots represent landslide locations and black dots indicate rock avalanches. Rock avalanches are landslides with > 1 million cubic meters of mass moved.

Future Work

We have identified a little over 2,000 landslides in Iceland, however, Iceland's landscape is constantly changing. We plan to continue updating the database and update run new analyses.

References

Bell, R., and T. Glade. "Multi-hazard analysis in natural risk assessments." Landslides 1 (2012): 1-10.

Evans, S. G. "Landslides." Bulletin of the Geological Survey of Canada 548 (2001): 43-79.

Guangrun, L. I. U., Y. A. N. Echuan, and L. I. A. N. Cao. "Discussion on classification of landslides."地质学报10.4 (2002): 339-342.

Highland, Lynn, and Peter T. Bobrowsky. The landslide handbook: a guide to understanding landslides. Reston: US Geological Survey, 2008.

Jakob, Matthias. "Landslides in a Changing Climate." Landslide Hazards, Risks, and Disasters, 2022, pp. 505–579.

Jakobsson, Sveinn P., Kristján Jónasson, and Ingvar A. Sigurdsson. "The three igneous rock series of Iceland." Jökull 58 (2008): 117-138.

Keefer, David K. "Rock avalanches caused by earthquakes: source characteristics." Science 223.4642 (1984): 1288-1290.

Malamud, Bruce D., et al. "Landslides, earthquakes, and erosion." Earth and Planetary Science Letters 229.1-2 (2004): 45-59.

Melosh, H. Jay. "The mechanics of large rock avalanches." (1987).

Nagler, T., et al. "Analysis of Landslides in Alpine Areas by Means of SAR Interferometry." IEEE International Geoscience and Remote Sensing Symposium.

NAKAMURA, Hiroyuki, and Satoshi TSUCHIYA. "Analysis on Piles for Landslide Control under Consideration to Displacement of Sliding Mass." Landslides, vol. 24, no. 2, 1987.

Nicoletti, Pier Giorgio, and Marino Sorriso-Valvo. "Geomorphic controls of the shape and mobility of rock avalanches." Geological Society of America Bulletin 103.10 (1991): 1365-1373.

Saemundsson, Th, Halldór G. Pétursson, and Armelle Decaulne. "Triggering factors for rapid mass movements in Iceland." Debris-Flow Hazards Mitigation: Mechanics, Prediction, and Assessment 1 (2003): 167-178.

WIECZOREK, G. F. (1984). Preparing a detailed landslide-inventory map for Hazard Evaluation and reduction. Environmental & Engineering Geoscience, xxi(3), 337–342.

Wong, H. N., K. K. S. Ho, and Y. C. Chan. "Assessment of consequence of landslides." Landslide risk assessment (1997): 111-149.

Van Den Eeckhaut, M., & Hervás, J. (2012). State of the art of National Landslide databases in Europe and their potential for assessing landslide susceptibility, Hazard and risk. Geomorphology, 139-140, 545–558.

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82.   Research Reflection by Claire Cruz

Claire Cruz

Faculty Mentor: Leif Anderson (Department of Geology)

I have been conducting undergraduate for two semesters now with Leif Anderson in the geology department. I have had the opportunity to expand my knowledge of ArcGIS, gain field work experience and learn more about geomorphology. The research I did during UROP has built off of topics learned in class and concepts I will use in my future career.

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83. Deep-Seated Gravitational Slope Deformation in the Sawatch Range of Colorado

Madeleine Festin

Faculty Mentor: JEffrey Moore: (Geology and Geophysics, University of Utah)

Deep-seated gravitational slope deformations (DSGSDs) are large-scale, creeping mass movements often involving entire mountain slopes. These enormous landslides may be on the order of kilometers in height and length but only move a few millimeters to centimeters a year. Secondary hazards are produced from frontal landslide failures, rock fall, and debris flows. While there have been many studies on DSGSD from the European Alps, few similar studies have been conducted in the United States. This research aims to identify DSGSD in a

2200 km2 area in the Sawatch range of Colorado, followed by mapping, geomorphic analysis, and comparison with satellite Interferometric Synthetic Aperture Radar (InSAR) displacement data. We used satellite imagery and Light Detection and Ranging (LiDAR) data to identify DSGSD features across the range, followed by a week-long field visit to collect ground data and imagery. Results show that DSGSD are relatively common across the Sawatch range, and many of these features are actively moving. We identified 28 sites that exhibit physical characteristics of DSGSD, of which eight appeared to be moving with a downward velocity of one to two centimeters per year. About half of the identified DSGSDs were west-facing. Almost all DSGSD occurred in granitic bedrock, with a few in metamorphic rock. The largest DSGSD was over 8 km2, but most fell between .5-1 km2. This research provides insights into their distribution and geomorphology of DSGSDs in the Sawatch range, while contributing to their broader understanding.

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84. An Individual Migration Story: Comparing Strontium Isotope Analysis in Enamel, Ivory, and Food Sources From A Single Modern Elephant

Katya Podkovyroff and Diego Fernandez (Geology and Geophysics)

Faculty Mentor: Diego Fernandez (Geology and Geophysics, University of Utah)

Abstract

Reconstructions of paleoclimate and paleoecology using stable isotopes of carbon, oxygen and strontium have become fairly common in many areas of the world. Focusing on strontium (Sr) isotope analysis, this project directly contributed to the collection, processing and data configuration of elephant ivory and molar samples as well as food eaten by said elephant. Misha, an elephant from the Salt Lake City Hogle Zoo, had previously been living in California and then was moved to Utah. To follow this migration, ivory, enamel, and food samples were analyzed to determine Sr isotopic composition. This data encapsulates the complexity and abilities in stable isotope analysis for migration research.

Introduction

The fields of archaeology and geochemistry have used stable isotope analysis for reconstructions of paleodiets and other prehistoric behaviors since the 1970s. These processes have been used as evidence for topics such as human evolution, migration research, transitions to agriculture, among other topics (Cerling et al., 2015; Eerkens, 2015). In particular, stable isotope analysis of tooth enamel, bone, and ivory has emerged as a powerful tool for migration research of living and fossil animals (Zazzo et al., 2012; Wooller et al., 2021; Lazzerini et al., 2021). As such, isotopic variations of mammalian tooth enamel, particularly in carbon, oxygen, and strontium, can be further studied to analyze variations in these migration patterns, climate and diet as a means of further understanding archaeological questions as well as modern mammals (Reade et al., 2015; Zazzo et al., 2012). An ivory tusk is a modified upper

incisor that forms incrementally throughout the lifetime of an elephant both in length and girth. Therefore one of the greatest difficulties with ivory is assessing the non-homogenous incremental tissues and differences in turnover. For example, the earliest formed or distal end of the tusk is used in an elephant's daily activities and can be worn away throughout their lifetime whereas the proximal end is the most recently formed part of the tusk (Coutu et al., 2016). Understanding the growth rate of elephant tusks is certainly important for sampling methods but a critical part of understanding the bigger picture is obtaining multiple tissue samples from the same specimen, such as enamel from a molar. Mammalian tooth enamel forms incrementally from the top to the base of the tooth with isotope analysis being attributed to measuring diet ($\delta 13C$) and tracking water sources ($\delta 18O$) during tooth mineralization (Wang & Cerling, 1994). Focusing on tooth enamel has allowed researchers to avoid any diagentic alteration damage since this tissue has high resistance to such a process (Wang & Cerling, 1994). 87Sr/86Sr reflects the bedrock strontium that is made available in soils to plants. Therefore, this bioavailable strontium is affected by the extent and type of weathering that occurs from the bedrock to soil which can be ultimately detected in the enamel or ivory samples of organisms (Coutu et al., 2016). As such, isotopic variations of tooth enamel or ivory can be used to create isoscapes of geographic regions as well as further studied to analyze variations in migration patterns, climate and diet as a means of further understanding archaeological questions as well as modern mammals (Reade et al., 2015; Zazzo et al., 2012). Furthermore, evaluating these isotopic measurements requires a fundamental understanding as to how these elemental compositions within organisms can indicate correlation

between seasonality and the elemental abundance in a given environment via the elemental signature (Koch, 1989).

Methods

Sample Collection

Ivory: The ivory samples were collected using a micromilling device from Ivory piece #889. Four sets of samples were collected, identified as the 300s and 400s-600s series. The 300s samples milling settings were as follows: 50 µm depth-per-pass, 5 pass count, 250 µm/sec scan speed, 25 µm/sec plunge speed, and the length of sample taken per measurement was 10 mm. The 400-600s samples milling settings were as follows: 100 µm depth-per-pass, 5 pass count, 250 µm/sec scan speed, 25 µm/sec plunge speed, and the length of sample taken per measurement was 20.8 mm.

Enamel: The molar samples were already collected with a distinction of two techniques: C4 (micromill from Misha Rm3.5b enamel) vs C6 (conventionally sampled from Misha_Rm3.5 enamel).

Food: Food was provided in paper envelopes and labeled with contents.

Elemental Analysis of Trace and Major Elements

Ivory, Enamel, Food: All samples were diluted by a factor of 50, using the recipe 20 µL digest + 10 µL Indium Standard + 970 µL 2.4% ◇◇◇ directly into an autosampler plate. A calibration 3 curve was prepared from single-element standards (Inorganic Ventures, Christianburg, VA, US), with indium added as internal standard. Diluted samples and calibration solutions were run in a triple quadrupole inductively coupled plasma mass spectrometer (ICPMS, Agilent 8900, Santa Clara, California, USA). Certified reference materials 1643f (Trace Elements in Water, National Institute of Standards and

Technology, Gaithersburg, MD, US) was used as a control for data quality.

Sr Purification and 87Sr/86Sr determination

Ivory, Enamel, Food: Using the Sr concentration calculated from the elemental analysis, aliquots containing 200 ng of Sr in 2 M HNO3 (prepared by mixing sample digest + conc ◇◇◇ + water) 3 and run through an automated Sr-purification system (PrepFAST, ESI, Omaha, NE, US). Purified Sr fractions, contained in 6 M HNO3, were then dried down at 185 ℃ and redissolved with 1mL of 2.4% ◇◇◇ . These Sr fractions were used for isotopic analysis in a multicollector ICPMS 3 (Neptune, ThermoFinnigan, Breman, Germany). Certified reference material 987 (SrCO3, National Institute of Standards and Technology, Gaithersburg, MD, US) was used to monitor the accuracy of the method

Results

Figure 1 displays 87Sr/86Sr of the ivory tusk. Each ivory series was run separately and the transition from California to Utah is better seen in the combined 400-600 series, likely due to the increased number of samples collected. California 87Sr/86Sr values are seen on the lower values (as seen on the right of both 300 and 400 series graphs) and Utah 87Sr/86Sr are seen on the higher values (as seen on the left of both series graphs). Interestingly there are two humps on the graphs but the more prominent one is likely to be the transition from California to Utah. Likewise, the laser ablation Sr isotope profile as seen in Figure 2, the graph begins with the Utah values on the left and then can be seen seemingly increasing (although the values on the vertical axis are in descending order moving up).

Figure 1. Micromill Sr isotope profile data from Misha Ivory piece #889

Figure 1. Micromill Sr isotope profile data from Misha Ivory piece #889

Figure 2. Previous Laser Ablation Sr isotope profile
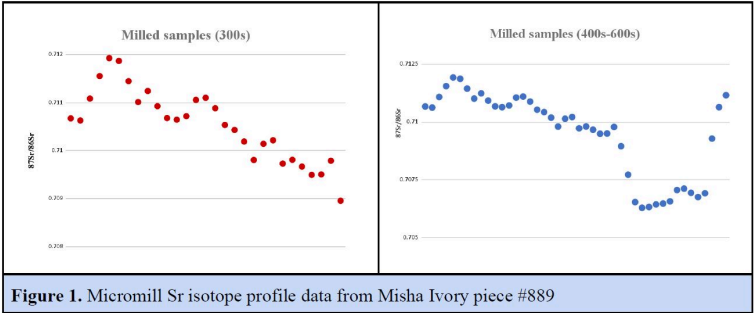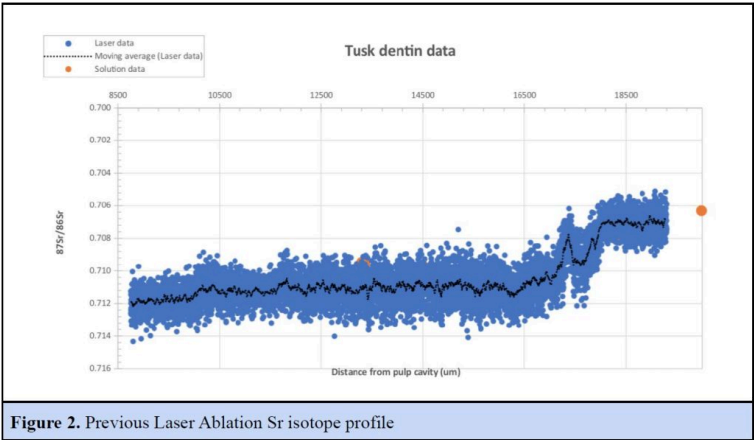
Enamel MC-ICP-MS was used in order to complete a Sr composition analysis of the enamel (as seen in Figure 3). The differences between the sample collection techniques are not stark and both show similar transition state values.
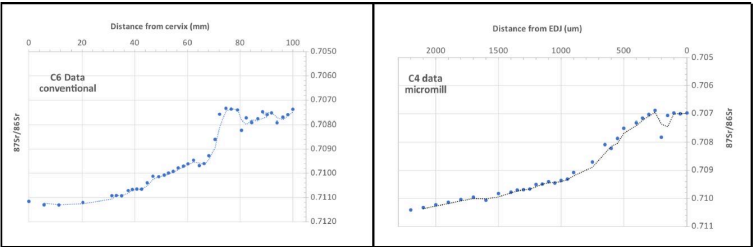
Figure 3. Sr isotope profile from C6 vs C4 Misha enamel

Food MC-ICP-MS was used in order to complete a Sr composition analysis of the food provided from the Hogle Zoo caretaker of the elephant (as seen in Figure 4). The sample types include grass hay, elephant hay, elephant pellets, elephant supplement (denoted as E. Supplement), bison hay, bison pellets, barn alfalfa, and other types of hay that were differentiated from the rest. Each sample was run twice and then the average was taken. The dates of when the food was used span from December 2000 to May 2012. Misha was moved to the Hogle zoo in April 2005 and died in September 2008.
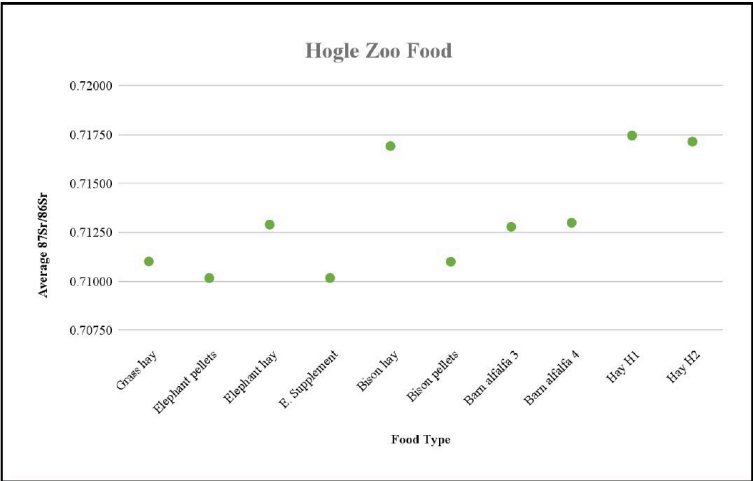
Figure 4. Sr isotope profile from Hogle Zoo food

Discussion and Future Results

Comparing the previously run tusk samples from the Laser-Ablation method done by Dr. Kevin Uno, former PhD student at the university, to the Dissolution method I completed, there was a confirmation of the previous results. Likewise, both the enamel analyses line up with one another as well as conceptually. When thinking of how samples were collected or presented, the distance from the pulp cavity of the molar was the innermost samples, where Utah data would be seen, versus moving to the outer regions which are older pieces of ivory that were formed when Misha was in California. Finally, the value of having this food data is to get an idea of how food intake can play a role in Sr isotope analysis, not just for carbon isotopes. In order to correct for mixing ratios of the food, an intake model will need to be made to evaluate how much Sr intake is being consumed by those food items.

Although this project is ongoing, the data collected up to this point is substantial. The immediate next steps include evaluating a molar sample from Misha and completing a Laser-Ablation Sr analysis to be compared to the other enamel data. The final product of this project will result in a comprehensive modeling of Sr turnover, the time it takes for an animal to switch values in Sr based on movement, which will be a major contribution to paleodiet research. Completing a multiple tissue, multiple method analysis of an animal that is relatively controlled as well as having data on the food the animal consumed can immensely assist in how to complete paleontology migration research in the future.

References

Cerling, T. E., Andanje, S. A., Blumenthal, S. A., Brown, F. H., Chritz, K. L., Harris, J. M., Hart, J. A., Kirera, F. M., Kaleme, P., Leakey, L. N., Leakey, M. G., Levin, N. E., Manthi, F. K., Passey, B. H., & Uno, K. T. (2015). Dietary changes of large herbivores

in the Turkana Basin, Kenya from 4 to 1 Ma. Proceedings of the National Academy of Sciences, 112(37), 11467-11472.

Eerkens, J. W., Sullivan, K., & Greenwald, A. M. (2016). Stable isotope analysis of serial samples of third molars as insight into inter- and intra-individual variation in ancient diet. Journal of Archaeological Science: Reports, 5, 656–663. https://doi.org/10.1016/j.jasrep.2015.11.003

Higgins, P., & MacFadden, B. J. (2004). "Amount Effect" recorded in oxygen isotopes of Late Glacial horse (Equus) and bison (Bison) teeth from the Sonoran and Chihuahuan deserts, southwestern United States Palaeogeogr. Palaeoclimatol. Palaeoecol., 206 (3–4) (2004), pp. 337-353. https://doi.org/10.1016/j.palaeo.2004.01.011

Koch, P.L., Fisher, D. C., & Dettman, D (1989). Oxygen isotope variation in the tusks of extinct proboscideans: A measure of season of death and seasonality. Geology 1989;; 17 (6): 515–519. https://doi.org/10.1130/0091-7613(1989)017<0515:OIVITT>2.3.CO;2

Lazzerini, N., Balter, V., Coulon, A., Tacail, T., Marchina, C., Lemoine, M., Bayarkhuu, N., Turbat, Ts., Lepetz, S., & Zazzo, A. (2021). Monthly mobility inferred from isoscapes and laser ablation strontium isotope ratios in caprine tooth enamel. Scientific Reports, 11(1), 1-11.

Reade, H., Stevens, R. E., Barker, G., & O'Connell, T. C. (2015). Tooth enamel sampling strategies for stable isotope analysis: Potential problems in cross-method data comparisons. Chemical Geology, Volume 404, pp. 126-135. https://doi.org/10.1016/j.chemgeo.2015.03.026.

Wang, Y., & Cerling, T. E. (1994). A model of fossil tooth and bone diagenesis: Implications for paleodiet reconstruction from stable isotopes. Palaeogeography, Palaeoclimatology,

Palaeoecology, 107(3-4), 281–289. https://doi.org/10.1016/0031-0182(94)90100-7

Wooller, M. J., Bataille, C., Druckenmiller, P., Erickson, G. M., Groves, P., Haubenstock, N., Howe, T., Irregeher, J., Mann, D., Moon, K., Potter, B. A., Prohaska, T., Rasic, J., Reuther, J., Shapiro, B., Spaleta, K. J., & Willis, A. D. (2021). Lifetime mobility of an Arctic woolly mammoth. Science, 373(6556), 806-808.

Zazzo, A., Bendrey, R., Vella, D., Moloney, A. P., Monahan, F. J., & Schmidt, O. (2012). A refined sampling strategy for intra-tooth stable isotope analysis of mammalian enamel. Geochimica Et Cosmochimica Acta, 84, 1–13. https://doi.org/10.1016/j.gca.2012.01.012

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85. **Research Reflection by Katya Podkovyroff**

Katya Podkovyroff

Faculty Mentor: Diego Fernandez (Geology and Geophysics, University of Utah)

As a second-bachelor's student graduating in Spring 2023 in Biology with a minor in Earth Science, I have a particular interest in paleobiology and biogeochemistry. Throughout my time at the University of Utah, I have had the opportunity and privilege to work in 2 different labs with 3 major projects. This particular submission highlights the work I have done since Fall 2021 in Dr. Diego Fernandez's ICP-MS Metals and Strontium Isotope Facility in the Geology and Geophysics Department at the University of Utah. Having these research experiences opened my eyes to the complexity of reconstructing paleoenvironments and studying paleobiological systems. I have also further developed my laboratory training and field work experience across my

multiple research projects, which was an essential element for my applications to graduate school, which I hope to begin Fall 2023. Being involved in the ICP-MS lab also led to my opportunities to become a UROP scholar with an independent research project and has opened doors to presenting research, attending conferences, and spurring my involvement in a senior thesis on a separate. All of these excellent opportunities have prepared me for my future goals of wanting to go into research and academia as well as procuring the foundation of the independent research process of conceptualizing unique research questions, formulating hypotheses and presenting findings.

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86. Grallator Tracks as a Window to Growth Strategies of Small-Bodied Theropods

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Because the soft tissue of non-avian dinosaurs rarely

preserves, studies of their ontogenies have been limited largely to skeletal remains. Fossil tracks are far more abundant than skeletal remains and record both locomotory information and impressions of the soft tissue that covered the feet. Thus, they have large sample sizes and can act as proxies for determining growth regimes among living dinosaur feet. The St. George Dinosaur Discovery Site at Johnson Farm (SGDS) in St. George, Utah, USA, has a wealth of tracks preserved on closely-spaced bedding planes. Thus, the site is as close to population sampling as possible. In particular, the SGDS contains a wealth of tridactyl *Grallator*tracks, which most likely were made by early, small theropods. Tracks were measured using eleven different linear metrics, which were then used to determine whether these early theropods experienced allometric or isometric growth of their feet, and evaluate the degree of individual variation. Analysis of track metrics using bivariate plots and regressions suggests strong, negative allometric linear relationships between the width of the track and the length of the track digits, as well as between anterior width and digit length. Linear relationships between R (distance from the middle of the fourth pad on digit IV to the second pad on digit III) and both total track width and the width between the claws on digits II and IV display more scatter, though they also indicate negative allometry. Independent of size, there appears to be significant variation in the angle between digit II and IV (divarication), suggesting that divarication angle is significantly influenced by substrate and/or locomotory behavior. All measurements in the dataset suggest a single continuous sample, suggesting that the tracks were likely made by a single species or closely related species having similar foot morphologies. These data suggest that as early small theropods increased in size, growth in toe length was slower than growth

in foot width. This could indicate that growth progressed to convey larger theropods greater stability to support their increased mass.

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SECTION X

Nursing

87. **Correlating Demographic Data with Preferred Learning Styles to Improve Neonatal Resuscitation Training for Guatemalan Lay Midwives**

Ella Baker

Faculty Mentor: Lauri Linder (Nursing, University of Utah)

ABSTRACT

Midwives throughout the world practice in vastly different geographic areas, socioeconomic contexts, and with a variety of resources. Though midwives attend the majority of births across the globe, their role and educational opportunities are not standardized. Training in crucial skills, such as neonatal resuscitation, is often inadequate or inaccessible to midwives with limited literacy in developing countries, such as Guatemala. While the Guatemalan government has offered midwifery training since 1955, the training has failed to change midwife knowledge because they are taught with written materials in Spanish, even though many midwives have limited literacy and speak Mayan dialects. More information is needed about how midwives best learn. This project aims to explore the learning styles of midwives in the context of neonatal resuscitation training with a larger goal of ultimately decreasing neonatal mortality. The project was conducted at Refuge International Health Clinic in San Raymundo, Guatemala, which is a suburban area located approximately 30 kilometers from Guatemala City. The project included 12 midwives with 1 to 46 years of midwifery practice experience. Participants reported a range of literacy from none to an ability to read and write in Spanish, and education levels ranged from elementary school through university. By means of a select-all-that-apply question added to a general demographic survey, the preferred

learning styles of the lay midwives were assessed. Response options to the question, "What methods best serve your learning?" included color drawings, black and white drawings, verbal instruction, demonstration, storytelling, written instruction, hands-on practice, verbally repeating what you learned to someone else, acting out what you learned, writing down what you learned, and/or watching a video. Participants could select all methods that applied. The work of this pilot study provided several insights into how this education can be better suited to learner needs. First, more color drawings should be included in distributed written materials. Color drawings were far preferred to black and white drawings by those included in the pilot study. Next, for indigenous participants whose first language is not Spanish, administrators should consider having Kaqchikel translators available, or avoid the use of complex medical terminology if translators are unavailable. From the findings of this study, it is clear that learning is a collaborative process. Program facilitators should regularly evaluate learner preferences and attempt to apply them as possible.

INTRODUCTION

Lay midwives are invaluable figures within maternal and infant health care, and especially so in developing countries. In fact, one study found that "Midwifery-led continuity of care is associated with superior outcomes for women and babies,

relative to other models of care" (Hewitt et al., 2021). Despite being essential to the well-being of birthing people, many lay midwives have limited literacy and lack the educational resources that might improve the quality of their care. These birth attendants are highly skilled, but training is often inadequate or inaccessible to low-literacy midwives in developing countries. One notable educational deficit lies in neonatal resuscitation training for lay midwives. While neonatal resuscitation is only necessary in 5-10% of all births, success in lay midwives providing this intervention is not shown in data from developing countries (Wall et al., 2009). In fact, 98% of worldwide neonatal deaths occur in these nations (Carlo et al., 2010). A gaping divide unfortunately persists between desired, and frankly, achievable, neonatal outcomes and the current reality within these countries.

A notable demographic variation also is evident in this population of care providers. Midwives practice in vastly different geographic areas and socioeconomic contexts, and with a varying range of resources available to them. Though midwives practice worldwide, attending the majority of births throughout time and across the globe, their role and educational opportunities are not standardized. While more defined educational standards for midwives are becoming widespread in developing countries, inequities still remain in implementing these standards across nations (Castro Lopes et al.,

2016). Quality improvement efforts are not universal, despite a pronounced need. Thus, tailoring the training programs to the unique needs of each group of lay midwives is important. Evaluating traditional teaching methods for lay midwives and revising these methods accordingly is critical to addressing infant and maternal health in developing countries. In turn, respecting diverse learning styles will maximize the educational hours of the trainees, trainers, and will improve the outcomes of such programs. Further emphasis should be placed on building a neonatal resuscitation curriculum that is both accessible and retainable.

Relevant Literature

According to the literature, quality improvement is becoming a greater focus for midwives across the world (Markaki et al., 2019). Midwives are crucial figures in this quality improvement process, as they are strategically situated to make measurable change within the field of women's and neonatal health. Within Guatemala, lay midwives are present at approximately 80% of indigenous births, and are poised to provide a distinct perspective of the reality within these settings (Lang & Elkin, 1997). Also, many of the births attended by midwives are outside of a hospital, as a recent study found that only half of indigenous women in Guatemala birthed at a designated healthcare facility (Juarez et al., 2020).

Despite their strong presence at most indigenous births, the majority of Guatemalan midwives' training is through apprenticeships administered by more seasoned lay midwives (Juarez et al., 2020). Unfortunately, more formal trainings offered to Guatemalan lay midwives by the government are written and in Spanish, despite the low-literacy level of many members of this population (Garcia et al., 2018). Little improvement in maternal or infant health outcomes has been shown by these trainings, which have been held monthly by the Guatemalan Ministry of Health since 1955 (Lang & Elkin, 1997). Within other developing countries, neonatal resuscitation curriculum is also shown to be inadequate. One study assessed the efficacy of the World Health Organization (WHO) Essential Newborn Care Course (ENCC) across six developing countries including Guatemala. Of the 62,366 infants included in the trial, no significant decrease in neonatal mortality from baseline was reported after the birth attendants received the WHO ENCC training (Carlo et al., 2010).

Consulting midwives is essential when evaluating the efficacy of administered training, especially in developing countries. One qualitative, narrative inquiry of thirteen Tanzanian midwives regarding reducing incidents of birth asphyxia concluded that "Midwives' stories are key to understanding their experience, and engagement, listening, interpretation, and understanding can

lead to a dialectical relationship between the researcher and research subject" (Becker et al., 2022). A conclusion that can be drawn from this statement is that both researchers and learners should adopt a growth mindset and work collaboratively to change the content of such curriculums for the better.

This idea reflects an anthropological concept from Madeleine Leininger's Theory of Transcultural Nursing, which is employed to promote cultural awareness and sensitivity (Murphy, 2006). According to the theory, emic knowledge is derived from the local culture, and is generally preferred over etic knowledge, which encompasses the researcher's perspectives and external ideas (Murphy, 2006). Understanding lay midwives and their history, before bringing in outside concepts, stands to improve the way neonatal resuscitation is taught.

No universal approach exists to address this community of practitioners, which is why demographic information and data on learning styles are key to adjusting the curriculum. In fact, organizations such as the Ministry of Health concur that midwifery education must be adjusted to fit the needs of each individual developing country to best enhance infant and maternal outcomes (Markaki et al., 2019). In these countries, midwives may be the only figures that stand between optimal and catastrophic outcomes.

Within nations like Guatemala, midwives are trusted and revered, and in many instances, they are a woman's primary contact for pregnancy-related complications (Glei et al., 2003). The time to comprehend their unique needs is now.

Purpose

This project aims to address why neonatal resuscitation training is not always translated into practice and decreasing neonatal mortality by communicating directly with a group of lay midwives with limited literacy in Guatemala during their own training on neonatal resuscitation techniques. By means of a select-all-that-apply question added to a general demographic survey, the preferred learning styles of the birth attendants themselves will be assessed. The optimal outcome would be to utilize this information to revise neonatal resuscitation programs in the future to make the training more effective and engaging, and improve retention of the learners. The specific aim of this thesis is to examine which educational methods Guatemalan lay midwives with limited literacy prefer. By delving into the response to the learning styles question, the neonatal resuscitation curriculum could be revised to better accommodate learner needs moving forward. With the proper teaching methods, the birth attendants could improve neonatal outcomes and pass on what they learn to midwives who did not have the opportunity to directly receive the training.

METHODS

Ethics Statement

The University of Utah's Institutional Review Board granted the study an exempt status and an expedited review (IRB_00154182). Verbal consent, rather than written, informed consent, was obtained from participants due to the limited literacy skills of the participants. This pilot study was part of a larger, quantitative, exploratory study involving a single group to generate descriptive statistics. The larger study examined change in knowledge regarding neonatal resuscitation after a culturally sensitive teaching on the topic. Demographic data were collected for the larger study on only one occasion from a group of twelve lay midwives and the survey administered included a question on preferred learning styles.

Neonatal Resuscitation Training Design

The study was conducted at Refuge International Health Clinic in San Raymundo, Guatemala, which is a suburban area located approximately thirty kilometers from Guatemala City. This project was conducted in partnership with the local Ministry of Health and the nonprofit organization, Refuge International. Refuge International has a local board and has maintained three clinics in Guatemala for twenty years. For recruitment purposes, nurses from the Ministry of Health who hold monthly educational sessions with local lay

midwives informed the attendees about the details of this neonatal resuscitation training.

The inclusion criterion for the study was anyone who identified as a midwife. The exclusion criterion for the study was the inability to speak either Spanish, English, or the indigenous language, Kaqchikel. No other exclusion criteria were applied due to the unique learning opportunity provided and the long distance the twelve lay midwives traveled to attend.

Upon arrival at the clinic, coffee and sweet bread were served while the Principal Investigator (PI) explained the purpose of the study. Consent was verbally obtained after program facilitators gave a brief description of the study and participants were told they could leave at any time. Participants were informed that their participation in the training would be regarded as giving consent. No written consent was obtained due to the low-literacy level of many of the study participants.

Next, the participants filled out a demographic survey. The data were collected in the form of a brief, written survey administered by program facilitators. The facilitators assisted participants with low-literacy in providing their responses. The demographic data collected included age, ethnicity (Hispanic or Indigenous), preferred language (Spanish, English, or Kaqchikel), years of midwifery experience, literacy, education level (none, Primary School, Secondary School, High School, College), and whether or not they have had neonatal resuscitation training in the past, and a question on learning styles that was structured as follows:

"What methods best serve your learning? Select all that apply.

- color drawings

- black and white drawings

- verbal instruction

- demonstration

- storytelling

- written instruction

- hands-on practice

- verbally repeating what you learned to someone else

- acting out what you learned

- writing down what you learned

- watching a video

After the demographic survey was completed, a pre-test evaluating prior knowledge of neonatal resuscitation was administered. Then, a twenty-nine minute focus group was conducted to explore what the participants already knew about neonatal resuscitation and needs they may have regarding the topic. The focus group was employed to collect pre-existing, emic knowledge before program facilitators provided outside information about neonatal resuscitation.

Next, an evidence-based, verbal training was given by expert faculty from the University of Utah's College of Nursing with frequent practice

and repetition, for the duration of two hours. After the training, a post-test was given with three questions designed to assess curriculum usability, feasibility, and satisfaction with the neonatal resuscitation content.

To thank them for their participation, each participant received a backpack with neonatal resuscitation and birth supplies. Participants also received a laminated reminder card with colorful images to remind them of neonatal resuscitation priorities along with written steps on the back of the card to reinforce knowledge retention. After the completion of the day's training, participants and program administrators enjoyed lunch together.

Data Management and Analysis

The Principal Investigator (PI), Dr. Kimberly Garcia, who speaks Spanish and has been working with Guatemalan lay midwives since 2009, translated the surveys and provided information to me. The PI analyzed the demographic data, calculating the percentage of answers regarding learning styles.

RESULTS

Participants

The study sample was twelve, Spanish-speaking lay midwives in Guatemala. They each identified as either Hispanic or Indigenous, had different literacy levels, varying years of schooling and midwifery experience, and had a wide array of ages. Table 1 details the various demographic characteristics of the study population. The mean age of participants was 50.75 (range = 31-72 years). The mean number

of years of experience as a lay midwife was 15 (range = 1-46 years). Of the participants, approximately thirty-three percent (four out of twelve participants) reported limited literacy (range = no formal education to elementary education) and approximately sixty-seven percent (eight out of twelve participants) reported they could both read and write (range = elementary education to university level). Of the participants, approximately forty-two percent (five out of twelve participants) reported Indigenous as their ethnicity, and approximately fifty-eight percent (seven out of twelve participants) reported Hispanic as their ethnicity.

Study Sample Characteristics

Table 1. Overview of Demographics and Preferred Learning Styles of Pilot Study Participants

Age	Ethnicity	Years of Experience	Can Read and Write	Education Level	Survey Response To Preferred Learning Styles Question
70	Indigenous	40	No	Elementary	color drawings
57	Indigenous	40	No	Elementary	color drawings, demonstration
31	Indigenous	13	Yes	Elementary	color drawings
54	Indigenous	17	Yes	Elementary	color drawings
38	Indigenous	22	Yes	High	color drawings
70	Hispanic	42	No	None	color drawings, verbal instruction, demonstration, hands-on practice, verbally repeating what you learned to someone else, acting out what you learned

40	Hispanic	5	No	Elementary	color drawings, verbal instruction, demonstration, storytelling, hands-on practice, verbally repeating what you learned to someone else, acting out what you learned, watching a video
72	Hispanic	46	Yes	Middle	verbal instruction, writing down what you learned
60	Hispanic	10	Yes	High	verbal instruction, written instruction
37	Hispanic	12	Yes	High	color drawings, verbal instruction, hands-on practice, watching a video
37	Hispanic	1	Yes	University	verbal instruction

43	Hispanic	4		Yes	University	color drawings, black and white drawings, verbal instruction, demonstration, storytelling, hands-on practice, watching a video						

Table 2. Ethnicity, Literacy, and Preferred Learning Styles: Frequency and Percentages (*See key below table)

Ethnicity	Can Read and Write	A.	B.	C.	D.	E.	F.	G.	H.
Indigenous	No	2 (100%)	0	0	1 (50%)	0	0	0	0
Indigenous	Yes	3 (100%)	0	0	0	0	0	0	0
Hispanic	No	2 (100%)	0	2 (100%)	2 (100%)	1 (50%)	0	2 (100%)	2 (100%)
Hispanic	Yes	2 (40%)	1 (20%)	5 (100%)	1 (20%)	1 (20%)	1 (20%)	2 (40%)	0

* Table 2 Key

What methods best serve your learning? Select all that apply.

A. color drawings

B. black and white drawings

C. verbal instruction

D. demonstration

E. storytelling

F. written instruction

G. hands-on practice

H. verbally repeating what you learned to someone else

I. acting out what you learned

J. writing down what you learned

K. watching a video

Table 3. Top Three Overall Answers for the Twelve Participants

1.   Color Drawings- 9 out of 12 participants (75%)

1.   Verbal Instruction- 7 out of 12 participants (58.3%)

1.   Demonstration- 4 out of 12 participants (33.3%)

DISCUSSION

From the data, several conclusions can be drawn. The first notable finding was that all indigenous participants selected color drawings as a preferred learning style. Of all of the participants, 75% selected color drawings as a preferred learning style. Only one (Hispanic) participant selected black and white drawings as a preferred learning style, and that participant also selected color drawings as a preferred learning style. The clear preference for color drawings within the sample

is notable, especially in regard to indigenous participants. Of note, many of the educational resources typically provided to Guatemalan lay midwives by the Ministry of Health only include black and white drawings.

It is important to note that studies on multimedia have indicated that images with color may help to improve attention and memory (Dzulkifli & Mustafar, 2013). This indicates that this seemingly arbitrary detail may have a larger impact than expected on learner retention. While cultural considerations such as preferences for color are often disregarded by educational administrators, the simple inclusion of color in teaching materials may appeal to both indigenous and Hispanic participants and improve their retention.

The next finding illuminated by the results of this study was that while all Hispanic participants selected verbal instruction as a preferred learning style, none of the indigenous participants selected this option. This is significant because, though all Kaqchikel-speaking, indigenous participants reported they were fluent in Spanish, verbal instruction in Spanish may not be most suitable for their learning. In one study regarding Ministry of Health-led midwifery training, "Nearly all participants endorsed Kaqchikel-Spanish language barriers as a major determiner of the quality of the training sessions they attended" (Chary et al., 2013).

Medical jargon, complex enough in Kaqchikel, proved even more difficult to understand in Spanish, the indigenous participants' second language (Chary et al., 2013). As none of the participants in this thesis' pilot study cited verbal instruction as a preferred learning modality, it could be worthwhile to explore developing a neonatal resuscitation curriculum in Kaqchikel. If this is not possible, perhaps learning objectives could be explained in more common terms, and through physical demonstration. As much of indigenous lay midwives' training is completed through apprenticeship, convoluted medical terminology in their second language is likely not appropriate for the best learning outcomes.

The median number of preferred learning styles selected by Hispanic participants was four, whereas the median number of selections for indigenous participants was only one. This finding may further suggest the intimate relationship between language and teaching styles. With current teaching methods, it is difficult to separate the two, so alternative solutions to verbal instruction in Spanish should be sought. The results of this thesis seem to confirm the conclusions of much of the previous literature on Guatemalan lay midwives and their education. Cultural considerations and language barriers matter. It is time to account for them and propose solutions such as including more color drawings and content in Kaqchikel using

common terminology and demonstration as principal educational modalities.

Limitations

The key limitation of this study was the small sample size of participants. Only twelve lay midwives attended the training. This is a very minimal representation of the nation's midwifery workforce as the Guatemalan government recognizes over 22,000 traditional midwives (Janetsky, 2022). To provide a more comprehensive sample, more midwives from different areas in Guatemala should be included in the study.

Also, only a single instance of data collection occurred, at the beginning of the training, and the next opportunity for reassessment is nearly a year from the time of the original pilot study. Since the data were collected before the training was completed, the midwives may have had different opinions on what learning style works best for them after they received the training.

Implications for Nursing

A central principle of quality nursing practice is patient-centered care. In the same way that knowing a patient's unique needs and preferences, then incorporating them into the treatment plan is key to best practice, the same principle should be applied to learners. The results of this pilot study illuminate the necessity of treating lay midwives as individuals, rather than following a prescriptive and dated approach to educational training. These

findings reaffirm the necessity of cultural humility and a solid understanding of the importance of health literacy while educating lay midwives across the globe. Accounting for language barriers and cultural preferences has already been proven to empower patients. The same principle could be translated into educational strategies for those who care for patients. Furthermore, it has been proven that monthly governmental training is not meeting the educational needs of lay midwives. More global health grants that incorporate research targeted at understanding how to fortify traditional birth attendants' skills should be proposed.

Directions for Future Research

The most immediate follow-up study for this project would be to re-evaluate the retention levels of neonatal resuscitation protocol during the PI's next visit to Guatemala. The original participants could also revisit their original answers to the survey question on learning styles, to see what change has occurred over the course of a year. On a larger scale, future research into this topic may include a broader study of lay midwives across several developing countries with high rates of neonatal mortality. More participants should also be recruited to provide a more comprehensive portrait of traditional birth attendants across the globe and how they best learn.

Implications for Policy

An implication for policy that may be proposed

as a result of this project is to include more accommodations within Guatemalan governmental training for traditional birth attendants with limited literacy. Color drawings should be a mandatory component of any written material the lay midwives receive during their monthly training with the Ministry of Health. Furthermore, Kaqchikel translators should be, at the very least, readily available to answer lay midwife questions during these training sessions. It could also be worthwhile to explore developing a neonatal resuscitation curriculum in Kaqchikel.

Conclusion

Education for lay midwives in developing countries on neonatal resuscitation can be further optimized to improve outcomes and learner retention of the curriculum. In countries like Guatemala, where the neonatal mortality rate is already high, teaching those who can intervene and apply neonatal resuscitation techniques is vital. The work of this pilot study provided several insights into how this education can be better suited to learner needs. First, more color drawings should be included in distributed written materials. Color drawings were far preferred to black and white drawings by those included in the pilot study. Next, for indigenous participants whose first language is not Spanish, administrators should consider having Kaqchikel translators available, or avoid the use of complex medical terminology if translators are

unavailable. From the findings of this study, it is clear that learning is a collaborative process. Program facilitators should regularly evaluate learner preferences and attempt to apply them as possible.

REFERENCES

Becker, J., Becker, C., Oprescu, F., Wu, C. J., Moir, J., Shimwela, M., & Gray, M. (2022). Silent voices of the midwives: factors that influence midwives' achievement of successful neonatal resuscitation in sub-Saharan Africa: a narrative inquiry. BMC pregnancy and childbirth, 22(1), 39. https://doi.org/10.1186/s12884-021-04339-7

Carlo, W. A., Goudar, S. S., Jehan, I., Chomba, E., Tshefu, A., Garces, A., Sailajanandan, P., Althabe, F., McClure, E. M., Derman, R. J., Goldenberg, R. L., Bose, C., Krebs, N. F., Panigrahi, P., Buekens, P., Chakraborty, H., Hartwell, T. D., & Wright, L. L. (2010). Newborn-care training and perinatal mortality in developing countries. New England Journal of Medicine, 362(7), 614–623. https://doi.org/10.1056/nejmsa0806033

Castro Lopes, S., Nove, A., ten Hoope-Bender, P., de Bernis, L., Bokosi, M., Moyo, N. T., & Homer, C. S. (2016). A descriptive analysis of midwifery education, regulation and association in 73 countries: The baseline for a post-2015 pathway. Human Resources for Health, 14(1). https://doi.org/10.1186/s12960-016-0134-7

Chary, A., Díaz, A. K., Henderson, B., & Rohloff, P. (2013). The changing role of indigenous lay midwives in Guatemala:

New frameworks for analysis. Midwifery, 29(8), 852–858. https://doi.org/10.1016/j.midw.2012.08.011

Dzulkifli, M. A., & Mustafar, M. F. (2013). The influence of colour on memory performance: a review. The Malaysian journal of medical sciences : MJMS, 20(2), 3–9.

Garcia, K., Dowling, D., & Mettler, G. (2018). Teaching Guatemalan traditional birth attendants about obstetrical emergencies. Midwifery, 61, 36–38. https://doi.org/10.1016/j.midw.2018.02.012

Glei, D. A., Goldman, N., & Rodríguez Germán. (2003). Utilization of care during pregnancy in rural Guatemala: Does obstetrical need matter? Social Science & Medicine, 57(12), 2447–2463. https://doi.org/10.1016/s0277-9536(03)00140-0

Hewitt, L., Dahlen, H. G., Hartz, D. L., & Dadich, A. (2021). Leadership and management in midwifery-led continuity of care models: A thematic and lexical analysis of a scoping review. Midwifery, 98, 102986. https://doi.org/10.1016/j.midw.2021.102986

Janetsky, M. (2022, March 29). To save lives, midwives mix Mayan heritage with Western medicine. National Geographic. Retrieved January 2, 2023, from https://www.nationalgeographic.com/culture/article/to-save-lives-midwives-mix-mayan-heritage-with-western-medicine

Juarez, M., Juarez, Y., Coyote, E., Nguyen, T., Shaw, C., Hall-Clifford, R., Clifford, G., & Rohloff, P. (2020). Working with lay midwives to improve the detection of neonatal complications in rural Guatemala. BMJ Open Quality, 9(1), e000775. https://doi.org/10.1136/bmjoq-2019-000775

Lang, J., & Elkin, E. (1997). A study of the beliefs and birthing practices traditional midwives in rural Guatemala. Journal of Nurse-Midwifery, 42(1), 25–31. https://doi.org/10.1016/s0091-2182(96)00069-9

Markaki, A., Moss, J., Shorten, A., Selleck, C., Loan, L., McLain, R., Miltner, R., Patrician, P., Theus, L., Ferrer, L., Góes, F., Valenzuela-Mujica, M. T., Zarate-Grajales, R., Cassiani, S., & Harper, D. (2019). Strengthening universal health: development of a nursing and midwifery education quality improvement toolkit. Revista latino-americana de enfermagem, 27, e3188. https://doi.org/10.1590/1518-8345.3229.3188

Murphy S. C. (2006). Mapping the literature of transcultural nursing. Journal of the Medical Library Association : JMLA, 94(2 Suppl), E143–E151.

Wall, S. N., Lee, A. C., Niermeyer, S., English, M., Keenan, W. J., Carlo, W., Bhutta, Z. A., Bang, A., Narayanan, I., Ariawan, I., & Lawn, J. E. (2009). Neonatal resuscitation in low-resource settings: what, who, and how to overcome challenges to scale up?. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics, 107 Suppl 1(Suppl 1), S47–S64. https://doi.org/10.1016/j.ijgo.2009.07.013

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88. Usability of the Revised Color Me Healthy App in Children with Cancer

Sydney Gilliland and Lauri Linder (Nursing)

Faculty Mentor: Lauri Linder (Nursing, University of Utah)

Purpose: The purpose of this project was to evaluate the usability of the revised *Color Me Healthy* app from the perspectives of children with cancer and their parents.

Background: The gold standard for symptom reporting in healthcare is self-reporting. Verbal self-reporting is often difficult for children. Children may more accurately and confidently report their symptoms using an mHealth device. The *Color Me Healthy* app has been developed to facilitate

self-reporting in children with cancer. The initial feasibility and acceptability of the app has been demonstrated; however, children and parents also noted opportunities to improve the user interface to enhance its ease of use and perceived usefulness prior to future implementation.

Methods: This User Centered Design study was guided by the Technology Acceptance Model which indicates that Ease of Use and Perceived Usefulness influence the adoption of a given technology. Children (6-12 years of age) receiving cancer treatment and their parents were invited to participate in usability evaluations in which they were guided through the app and asked to complete specific tasks within the app. Completion of tasks were documented on a usability log and scored as "completed independently," "required a verbal prompt," or "required a physical prompt." Children and parents participated in brief interviews in which they were asked what they liked best about the app, what they liked the least, and suggestions for improvement. Parents also completed the Technology Acceptance Model Perceived Usefulness Scale (TAM PUS). Possible TAM PUS scores range from 4 to 24 with lower scores representing greater perceived usefulness. As 5 child-parent dyads completed usability evaluations, data were summarized and shared with the developer team to guide additional refinements.

Assessment: Fourteen racially, ethnically, and geographically diverse parent-child dyads participated. Children were a median of 8.5 years; 9 were boys; and 13 had leukemia. Parents were a median of 38.5 years of age, and 11 were mothers. After 3 cycles of usability evaluations, children and parents were able to complete key tasks independently, supporting its ease of use. Eleven children and 12 parents also indicated their preference for using the app as a method of symptom

reporting, supporting its perceived usefulness. Median TAM PUS scores were 7 across all 3 cycles and provided evidence that parents deemed the app useful to understand and respond to their child's symptoms.

Conclusion: mHealth devices, including the *Color Me Healthy* app provide promising methods of symptom self-reporting in children with cancer. Because nurses should encourage self-reporting tools whenever possible, mHealth tools may provide an approach to self-reporting that is perceived as safer and more familiar. The revised version of the Color Me Healthy app demonstrates ease of use and perceived usefulness. Future directions include evaluation of the clinical utility of the revised app to support initiation of symptom management interventions.

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89.   Pre-Loss Group Support (Plgs) for Family Care Partners of Persons Living with Dementia (Plwd): Perceptions of Nursing Home Administrators and Social Workers

Kimberly Ponce Gonzalez; Katherine Supiano (Nursing); and Laura Bradbury (Nursing)

Faculty Mentor: Katherine Supiano (Nursing, University of Utah)

Background

Project: Dementia family caregiving may span more than a decade and places many family care partners (CP) at risk for poor bereavement outcomes and a lack of preparation for the death of a person living with dementia (PLWD.) Prolonged grief disorder (formerly called Prolonged grief), is "a form of mourning characterized by yearning for the deceased, intrusive thoughts of the person who died, unrelenting sorrow, withdrawal from previous social relationships, difficulty accepting the death, and a sense that life is without purpose." (Newton et al., 2011; Simon, 2015; Crunk et al., 2017.) Prolonged grief has been found in approximately 20% of bereaved dementia family caregivers. Prolonged grief remains under-recognized, underdiagnosed, and undertreated in this population. Few efforts to address the prevention of prolonged grief have been identified. Therefore, this study is funded by the Alzheimer's Association to develop, evaluate, and implement PreLoss Group Support (PLGS). This is to prepare persons at high risk for prolonged grief, and facilitate a healthy grief process upon the death of their person with dementia. This is a pragmatic trial consisting of the training for nursing home social workers, and evaluation of the feasibility of their application of the PLGS intervention. PLGS is a 10-session multi-modal comprehensive psychotherapy, designed by Dr. Supiano, and intended to be administered by trained nursing home social workers (NH-SWs).

Stakeholders: Institutional "buy-in" is an essential component of pragmatic trials located in community settings. Administrators are well aware of, and concerned about family

caregiver needs–as it impacts the wellbeing of residents. Nursing home staff are often presented with 'complaints' by the family. Often these are legitimate complaints, but they may also represent displaced guilt, and merit provision of support to the family. Families who express guilt about nursing home placement are at risk for prolonged grief. These family members are of greatest concern to the leadership team of nursing homes. To assess concerns of nursing home administrators, I conducted semi-structured interviews with 5 nursing home administrators to acquire the "stakeholder" viewpoint on family caregivers. The questions for the interview were created with the collaboration of my mentor, and the questions will address the administrator and facility concerns about the family caregivers of nursing home residents living with dementia.

Social Workers: Access to efficacious group interventions for dementia family caregivers has been limited by geography, social isolation, and lack of trained clinicians. The added impact of COVID-19, with visitation restrictions in nursing homes, and increased risk of death in residents with ADRD calls for immediate evaluation of telehealth interventions. Telehealth delivery of both social worker training and Pre-loss Group Support (PLGS) addresses this gap. Nursing home SWs are ideally situated in the practice setting and have clinical expertise to learn and implement PLGS, allowing simultaneous evaluation of telehealth SW training acceptability, suitability for nationwide dissemination, and the impact of telehealth delivered PLGS. As the NH-SWs are undergoing the PLGS clinical training, I will assess the NH-SW satisfaction with the PLGS training, as part of our evaluation of the pragmatic trial.

Method

Stakeholders: I conducted semi-structured interviews with

5 partner nursing home administrators and acquired the "stakeholder" viewpoint on family caregivers of residents with dementia. Questions included; asking who the administrators were, why they worked at the nursing home, services they offered, how placement works, challenges and concerns they may face within their facility with families' preparation for a death of a PLWD. I conducted a qualitative analysis to synthesize NH administrator responses to interview questions.

Social Workers: Using professional actors, we created a simulated PLGS 10-session group as a series of training videos. The training utilized our Stage-3 PLGS Facilitator manual. And the training incorporated didactic content, interactive case examples, and pragmatic application concept of PLGS for telehealth delivery to CPs by NH-SWs. I interviewed and summarized training satisfaction surveys of our participant social workers as part of our evaluation.

Results

Stakeholders: After conducting interviews with the 5 administrators of the nursing homes and veteran homes, we have found administrators find it important that their PLWD be treated as a person. Administrators stated that they hope to have dementia seen as a disease, as something separate from the person. The disease does not define who the person is at the time they are diagnosed until the end of life.

Social Workers: The participant satisfaction surveys were done with a 7-item satisfaction scale with a range of 3-21, satisfaction rate was 95% (highly valuable), with 100% indicating they would recommend training to SWs in other facilities.

Conclusion

Nursing home administrators valued family CPs and wanted them treated with respect. My interviews with the nursing

home social workers showed that they valued the training and were eager to support the families using the Pre-Loss Group Support. These interviews have guided the next steps of implementation of the PLGS pragmatic trial. I gained multiple perspectives to viewing how underrepresented communities gain mental health resources and assistance. From a professional business viewpoint, the nursing home administrators realize that when the PLWD is viewed as a human being, apart from the disease and is treated as a person, the family CPs feel less guilt. They are better able to appreciate and honor the staff which leads to an overall better community in the nursing homes. From a clinician viewpoint, the social workers believe training in dementia and the needs of family CPs is highly valuable within the mental health field. Within both the NH administrator and social work perspectives, nursing home professionals find progressive education on the dementia trajectory is important for family CPs. Mental health professionals need to be equipped to guide families, because the grieving process begins when the PLWD loses ability to self-represent their needs, thoughts and feelings. My contribution to the project has advanced education within dementia, grief and loss. I learned in order to assist underrepresented communities we first need to understand the perspectives within them in order to gain a bigger understanding to helping those that need it in the future.

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90. **Research Reflection by Kimberly Ponce Gonzalez**

Kimberly Ponce Gonzalez

Faculty Mentor: Katherine Supiano (Nursing, University of Utah)

When I transferred from Salt Lake Community College to the University of Utah I did work-study with Caring Connections: A Hope and Comfort in Grief Program at the College of Nursing. There, the Director, Dr. Katherine Supiano offered me to assist her in her research grant she had been working on involving grief and loss and dementia. She suggested me to apply for the Undergraduate Research Opportunities Program (UROP) because she believed I had high capabilities and she had previous students that have done this program with her as well. I came in having little to no knowledge about research and how to apply it besides reading

literature reviews. This program has helped me immensely in achieving experience with research. And I have grown to love research so much that it reaffirmed why I love Psychology. It has also defined my choice in pursuing to be a Clinical Psychologist in the future. Dr. Supiano has been a wonderful mentor with guiding me, in which I am super grateful for, and I am looking forward to creating more research in helping underrepresented communities.

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91. Pediatric Oncology Patient's Conceptualization of Cancer Symptoms

Minahil Usman and Lauri Linder (Nursing)

Faculty Mentor: Lauri Linder (Nursing, University of Utah)

Background

According to American Childhood Cancer Organization [1], approximately 1 in 285 children in the U.S. are diagnosed with cancer before their 20th birthday. But treatments often involve intense and difficult procedures which can make children feel distressed while also disrupting their quality of life [2]. Their ability to participate in daily activities

becomes severely restricted and relationships with parents and siblings are also negatively affected as a result of the short- and long-term symptoms experienced [3].

Learning about how children perceive and/or describe their symptoms is challenging because they often become frustrated when asked to rate symptoms on a self-assessment scale or to tell individuals around them who can't truly appreciate or understand their suffering. Most children prefer to deal with their feelings on their own. However, it is important to discover how children feel and think about these symptoms because it can aid nurses, healthcare providers, and families to provide a more comprehensive, individualized, and sensitive approach to care. And because suffering with cancer is a shared experience, children's overall conceptualization of symptoms often constituted a multidimensional phenomenon of multiple symptoms, social interactions, and various environments. Therefore, to effectively track the course of the disease, it's vital to explore and recognize relationships on the intrapersonal, interpersonal, and transpersonal levels as all these impact the children's experiences with symptoms [2].

To address this problem, I plan to embark on a project where I will be reviewing transcripts of qualitative cognitive interviews conducted by Dr. Linder with child cancer patients as part of her KIDS-SM study on the online software program, Dedoose. I am hoping that utilizing excerpts of the transcripts as my primary source of analysis will help me gain a sense of the whole picture regarding children's experiences with the disease (i.e., how they describe aspects of symptoms). And then I can use a content analysis via an inductive qualitative approach

to identify commonalities among the excerpts and ultimately understand child cancer patient's conceptualization of symptoms.

Aims

1. Describe how children with cancer between the ages of 6 to 12 characterize their symptoms and expressions they use in relation to these symptoms.

Conclusion

A comprehensive approach to assessing symptoms in clinical settings is necessary to not only improve symptom management and quality of life for pediatric oncology patients throughout the trajectory of their disease, but also to understand illness-related distress and address the changing nature of symptoms daily [3]. Some children experience symptoms – what they often refer to as 'feeling states' – so often that it becomes their 'new normal.' However, these 'feeling states' can't be quantified on self-assessment scales; rather, we need to consider adopting a multi-faceted approach that studies symptoms, social interactions, and environments altogether to be able to fully capture the subjectivity of the suffering and experiences children attach to their symptoms [2]. And this is exactly what I hope to accomplish through my research project.

References

1. US Childhood Cancer Statistics. (n.d.). ACCO. Retrieved September 30, 2022, from https://www.acco.org/us-childhood-cancer-statistics/

2. Woodgate, R. L. (2008). Feeling states: a new approach to understanding how children and adolescents with cancer experience symptoms. Cancer nursing, 31(3), 229–238. https://doi.org/10.1097/01.NCC.0000305731.95839.ca

3. Linder, L. A., & Hooke, M. C. (2019). Symptoms in Children Receiving Treatment for Cancer-Part II: Pain, Sadness, and Symptom Clusters. Journal of pediatric oncology nursing : official journal of the Association of Pediatric Oncology Nurses, 36(4), 262–279. https://doi.org/10.1177/1043454219849578

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92.   **Research Reflection by Minahil Usman**

Minahil Usman

Faculty Mentor: Lauri Linder (Nursing, University of Utah)

This was my first time working on a research project from start to finish, and I couldn't have asked for a better mentor to guide me through every step of the process. I started this research as part of my Honors thesis, but it quickly became more than that. In addition to helping me advance my research skills, it also helped me further my passion for medicine and decide what specialty I wanted to pursue. I always knew I loved being around and working with children, but I didn't realize that it could be something I could do for the rest of my life or that this project would have such a big impact on my life at all. Cancer patients are especially close to my heart because of my experience working with them as a camp counselor at Camp Hobe and because my grandmother suffered from leukemia. So, understanding what their symptoms meant to

them was something I was very passionate about, as it could help improve their care and possibly also extend their life expectancy. Every time I picture a child, I picture them happy, playing with toys, running around the block, laughing, etc. And to think that some children can't have the same experience as others due to a God-given illness is just so heartbreaking to me. But at least we can perform research studies like this one and play a role in trying to give them a chance at a somewhat normal life.

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SECTION XI

Science

93.   **Spectroscopic Studies of Lanthanide Nitrides**

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Faculty Mentor: Michael Morse (Chemistry, University of Utah)

With the employment of Resonant Two-Photon Ionization (R2PI) spectroscopy and jet-cooled molecular beams, the sharp predissociation thresholds of lanthanide metal nitride diatomics (LnN, Ln = Pr, Nd, Gd, and Tb) were measured. These species do not have previously reported bond dissociation energy (BDE) measurements in the literature which prevents the classification of important periodic trends and overall fundamental characteristics. One of the most informative fundamental characteristics of a molecule is its BDE, which

is the amount of energy required to bring a molecule from its ground vibronic state to its ground separated atom limit. The first portion of the R2PI spectra illustrates a congested manifold of molecular vibronic states at energies below the ground separated atom limit. The second portion of the spectra is resolved when the molecule of interest is excited to energies at or above the ground separated atom limit. Here, the molecule rapidly dissociates and the measured ion signal drops to a baseline indicating zero molecular signal. The BDE can be accurately and precisely assigned at the transition from molecular signal to baseline. The BDEs of the LnN studied in this work are D0(PrN) = 5.362(3) eV, D0(NdN) = 4.820(3) eV, D0(GdN) = 4.273(3) eV, and D0(TbN) = 4.261(3) eV. These results help illuminate the fundamental principles of the chemical bonds between these lanthanide metals and nitrogen.

Predissociation Threshold in $^{141}Pr^{14}N$

Predissociation Threshold in $^{142}Nd^{14}N$

$^{142}Nd^{14}N^+$ signal

$D_0(NdN) = 38876(25)$ cm^{-1}

$= 4.820(3)$ eV

$^{186}W^+$ signal

Ion Signal

Wavenumber (cm^{-1})
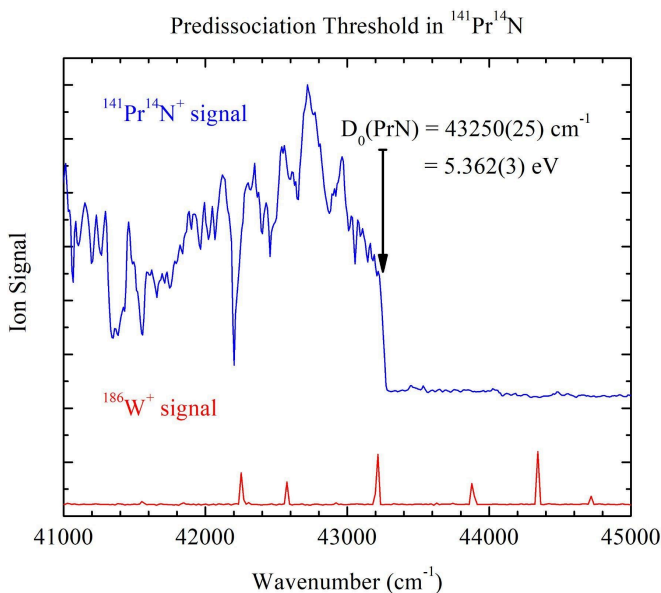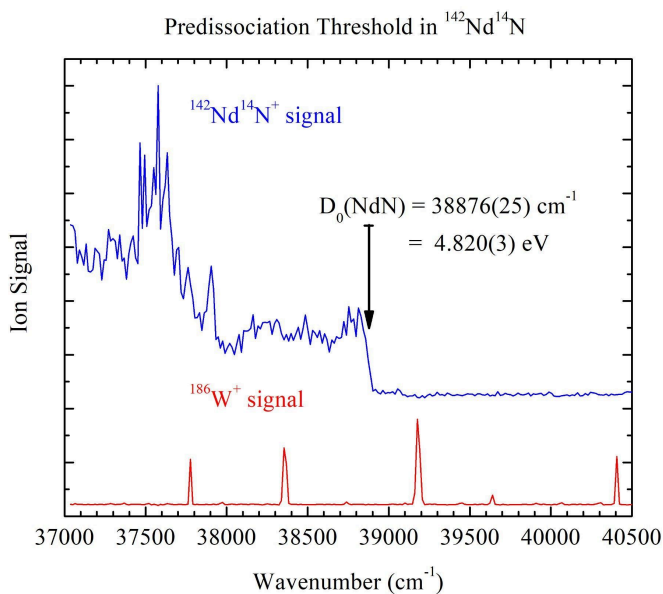
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94. Feasibility of Triphos-Ligated Cobalt as an Electrocatalyst for Carbon Dioxide Reduction

Lillee Casselman and
Caroline T. Saouma
(Chemistry)

Faculty Mentor: Caroline T. Saouma (Chemistry, University of Utah)

In the wake of increasing concern and awareness surrounding global warming, there is a strong push to not only reduce carbon dioxide emissions, but also to advance technologies that negate emissions. One plausible way to do this is through conversion of carbon dioxide to fuels or fuel

precursors (carbon monoxide, methanol, formic acid). Very few catalysts can convert carbon dioxide to methanol, one of which is triphos-ligated cobalt; this species has been shown to hydrogenate carbon dioxide to methanol under elevated temperatures and pressures. Knowing that the same catalyst can electrocatalytically reduce protons to hydrogen gas, my project is focused on determining if triphos-ligated cobalt can also serve as an electrocatalyst for carbon dioxide reduction. Using very weak acids, we can circumvent hydrogen gas production at mild potentials, and access a catalytic current that is pronounced in the presence of both carbon dioxide and protons. After synthesizing and characterizing suspected intermediates in the catalytic cycle, my current research efforts have shifted towards controlled-potential electrolysis, and quantifying the resulting products when subjecting the triphos-ligated cobalt and a proton source to a negative potential under carbon dioxide. This work will be contextualized in terms of how carbon dioxide electrocatalysts relate to carbon dioxide hydrogenation catalysts, which may provide strategies for future catalyst designs.

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95.   **A Novel Organelle of the Synapse**

Gareema Dhiman

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During neurotransmission, neurons constantly fuse and recycle synaptic vesicles from the presynaptic membrane. Like other organelles, synaptic vesicles and proteins must be removed when damaged or overused. Build-up of damaged proteins may underlie neurodegenerative diseases like Parkinson's Disease. However, a pathway for removal of synaptic material is unknown. Common degradative pathways of neuronal cells include the autophagy, proteasome, and endo-lysosomal systems. Damage within any of these pathways leads to accumulation of waste at synapses.

Members of the Jorgensen lab have recently discovered a new organelle in *Caenorhabditis elegans* called a surveillant. The lysosome-derived organelle is found to probe synaptic spaces and take up synaptic proteins and aggregates. Surveillant numbers doubled when stressed using a 34° C heat shock for 4 hours. Surveillants share important similarities to lysosomes; however, surveillants are transported to and from synapses, unlike lysosomes, which reside in the cell soma. To better understand the origins of surveillants, I aim to answer the question, what is the molecular composition of surveillants? To address this, we have tagged synaptic vesicle proteins as well as autophagy and lysosomal proteins with fluorescent proteins to measure if they colocalize with surveillants. Identifying whether colocalization occurs is important in uncovering what pathway surveillants are involved in and how they can be differentiated from other degradative organelles. This research is important for broadening our understanding of how neurons respond to severe stress, with relevance to human diseases such as stroke, Alzheimer's, and Parkinson's Disease.

INTRODUCTION

As the likelihood of developing neurodegenerative diseases becomes more apparent with increasing life expectancies. Understanding the mechanisms involved in neurodegeneration is key to developing new treatment approaches (Alzheimer's Association, 2023). Neurodegenerative diseases are often characterized by accumulations of aggregated or misfolded proteins, leading to disrupted synaptic function (Monaco, 2020). For example, in Parkinson's disease accumulation of α-synuclein has been identified at neurons (Di Maio, 2016). Mounting evidence indicates that this accumulation leads to disruption of neurotransmission (Recasens, 2014). Protein aggregation at neurons may be more likely due to their unique physiology and morphology. Neurons are one of the most vulnerable cell types to protein aggregation (Kundra, 2020). Synapses

have a high turnover rate for protein, membrane, and degradative mechanisms (Wnukowski, 2023). The main degradative organelles of neurons are lysosomes, which break down proteins using hydrolytic proteases. Lysosomes are restricted to the soma, dendrites, and the axon initial segment (AIS), which can be far from the synapse (Edwards, 2013) (Figure 1).
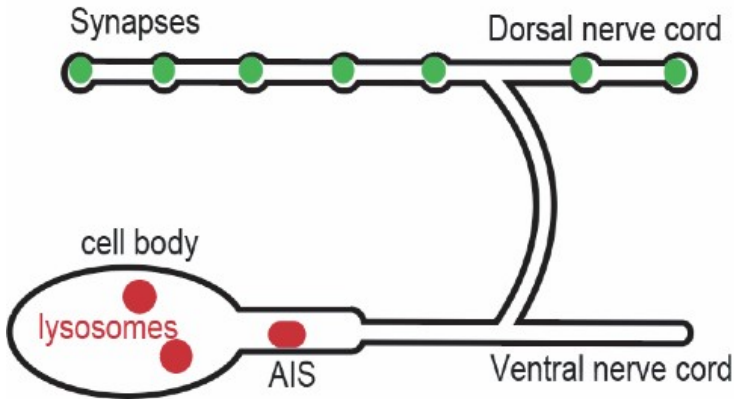
Figure 1: Lysosomes (red) are contained in the cell body and axon initial segment (AIS) in a *C. elegans neuronal cell.* At the synapses waste often builds up (green) that can lead to neurodegenerative diseases if not effectively degraded.

Proteins destined for degradation in the lysosome are transported by organelles such as late endosomes or autophagosomes. This allows for proper sorting and recycling of cellular components at synapses (Yu, 2018). During macroautophagy (generally referred to as autophagy) a double membrane forms around damaged proteins or other small organelles to form an autophagosome (Figure 2) (Yu, 2018). The autophagosomes are transported back along the axon and

eventually fuse with lysosomes. In addition to macroautphagy, damaged proteins and organelles as well as endocytic vesicles can be degraded through microautophagy, endosomal microautophagy, and chaperone-mediated autophagy. Another main degradative pathway is the proteasome/ubiquitin (Figure 2). E3 ubiquitin ligases recognize and attach ubiquitin sequences to damaged proteins, which are cleaved into small peptides in the proteasome. Failure of any one of these pathways may lead to an accumulation of waste and cellular debris at synapses (Yu, 2018). Determining the precise mechanisms by which synaptic vesicles and proteins are recycled is therefore critical in the context of understanding neurodegenerative disease.

Autolysosome

Release of
amino acids,
fatty acids

Cytoplasmic
material

Initiation
membrane

Docking and fusion

Initiation
membrane

Lysosome

Autophagosome

Endocytosis
& MVB pathway

Chaperon mediated
autophagy

Ub

Lysosome-
Signaling

PAS

Autophagy

Microautophagy

Current Opinion in Cell Biology
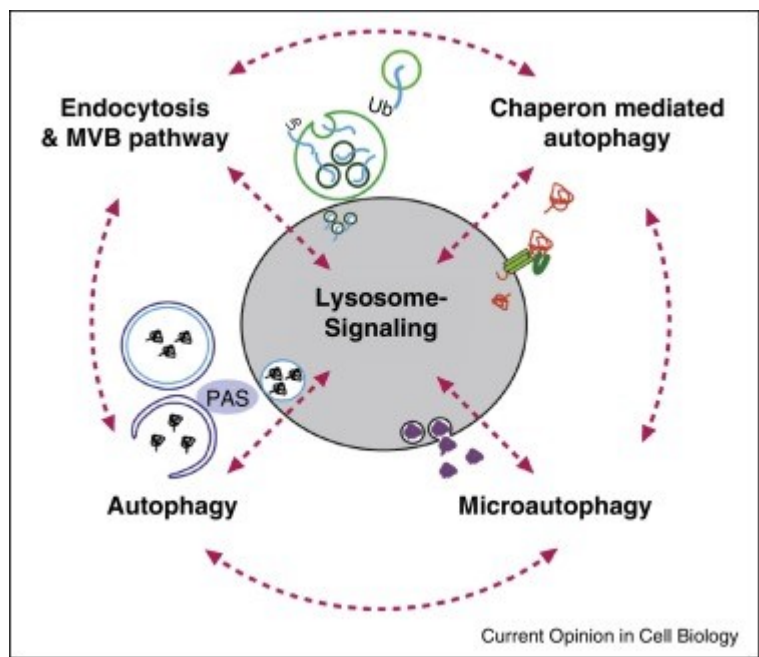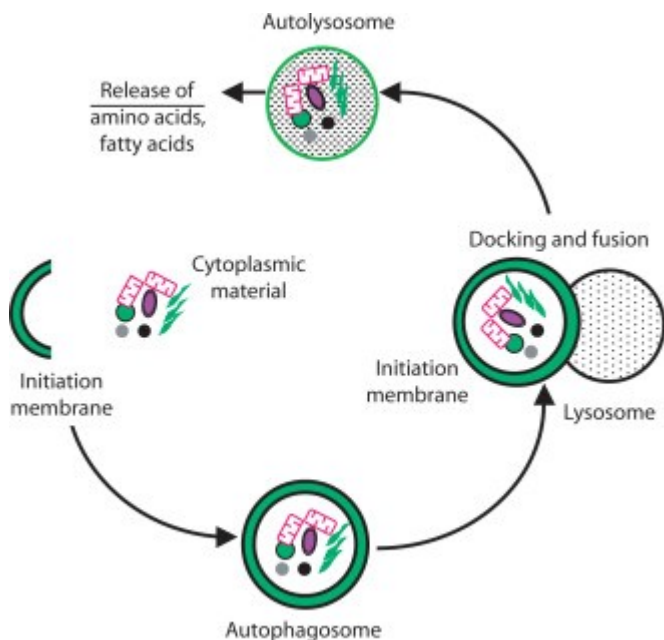
A   **B**
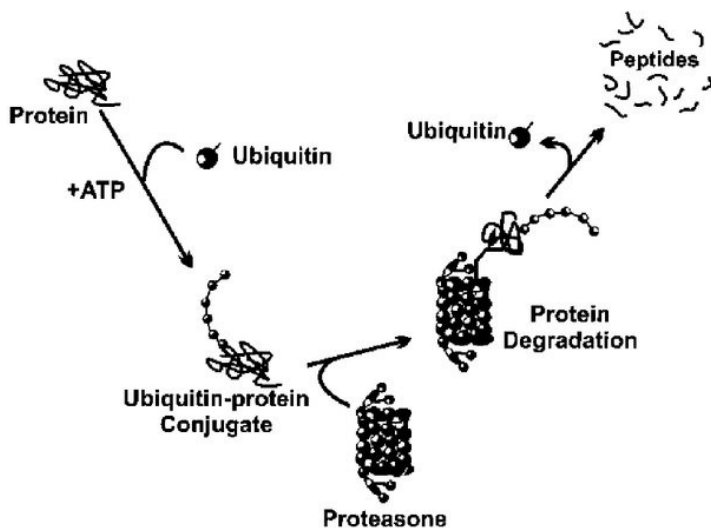
C

Figure 2:

(A) A closer depiction of the autophagy process from Wilfred, 2008. The process is initiated by the formation of a membrane that enlarges into a double membraned vesicle known as the autophagosome. This sequesters molecules and organelles. The autophagosome eventually fuses with a lysosome to form an autolysosome. The contents withing are degraded by acidic lysosomal hydrolase. **(B)** Overall pathways in which lysosomes are involved in from Lukas, 2016. Autophagy, endocytosis, multivescular bodies (MVB) pathway, chaperone mediated autophagy, and microautophagy are shown to be regulated by lysosomal signaling. **(C)** Basic proteasome pathway in protein degradation from Gheith, 2009. Proteins are first conjugated with ubiquitin by an ATP-

dependent reaction. The protein is recognized and degraded to peptides by proteasome.

Environmental stresses such as heat shock, hypoxia, and starvation can directly affect lysosome biogenesis and function in *C. elegans*. Heat shock increases the number and size of lysosomes in neurons. In addition to an increase in lysosomes, the Jorgensen lab has observed the formation of a unique organelle called a surveillant (Wnukowski, 2023). The surveillant is likely derived from lysosomes, but unlike the lysosome can leave the AIS to "surveil" synapses, taking up protein aggregates. We present observations of possible proteins that may make up the molecular composition or are being taken up by surveillants in the synapse through a degradative pathway. Surveillants serve as a unique organelle that may be arise as a response to environmental stressors when the normal degradation pathways are overwhelmed.

BACKGROUND *C. ELEGANS* AS A MODEL SYSTEM:

To determine the molecular components of surveillants, I performed studies using the nematode *Caenorhabditis elegans* (Figure 3).
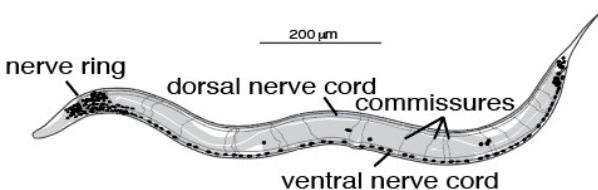
Figure 3: *Caenorhabditis elegans* diagram highlighting nerve cords and commissures.

C. elegans offer several advantages to work with to study surveillants: they require minimal labor in upkeep, have a rapid reproductive cycle (~3 days), and are self- fertilizing hermaphrodites. Males can be easily generated for genetic crossing of strains. C elegans are highly amenable to transgenesis and provides an ideal system to study co-localization of proteins due to its simple morphology, transparency, and annotated genome sequence (Sugi 2016). Additionally, many genes involved in autophagy, lysosomal degradation, and synaptic transmission are conserved from *C. elegans* to humans, which provides a simple model to understand the basic mechanisms of lysosomal function in the context of human diseases (Sugi, 2016).

LYSOSOME LABELING

LMP-1 (an ortholog of human LAMP2 [lysosomal associated membrane protein 2]) is a known marker of lysosomes and an abundant protein within the lysosome membrane (Settembre, 2013). LMP-1 can also be found on endosomes and transport organelles (Cheng, 2018). Generally, it can be difficult to label lysosomes due to the ambiguity in the definition of a lysosome. Some studies call all Lamp-1 labeled organelles lysosomes, while others describe lysosomes based on luminal pH (Liao, 2019; Bucci, 2000). Due to the low expression of lysosomes in neurons, knowledge regarding degradation through this pathway at synapses is scarce. Additionally, it can be difficult to assess whether lysosomes experience anterograde motion to axonal and synaptic spaces. In GABA neurons, the *C. elegans* kinesin adaptor UNC-16 restricts the transport of lysosomes

past the AIS, blocking them from reaching the axon and synapse (Ferguson, 2017).

The Jorgensen lab has defined an organelle called a surveillant, which has lysosome like properties, but undergoes anterograde transport to axonal and synaptic spaces in neurons. To study surveillants by microscopy, single copy insertions of lysosomal and synaptic proteins fused to fluorescent proteins were expressed in *C. elegans* GABA neurons, driven by the *unc-47* promoter. GABA neuron cell bodies are located in the ventral cord but send axons to the dorsal cord. By imaging only fluorescent proteins in the dorsal cord we can distinguish these organelles from somatic lysosomes.

LGG-1

LGG-1 within *C. elegans* enables GABA receptor binding and ubiquitin protein ligase binding. It is also known as an autophagosome marker which has been used throughout this experiment. Using LGG-1 has allowed for colocalization experiments with surveillants to be done and further understand how the organelle differs from autophagosomes.

SURVEILLANTS

Using a tandem fluorescent reporter, surveillants are described as mCherry::LGG-1 labeled organelles in the dorsal cord and are differentiated from autophagosomes by a quenching of eGFP::LGG-1 (Figure 4). Under acidic conditions, eGFP is degraded in lysosomes, whereas mCherry is acid resistant, suggesting surveillants are acidified like lysosomes. mCherry::LGG-1 organelles can bud off somatic lysosomes, suggesting these are surveillants of a lysosomal origin.
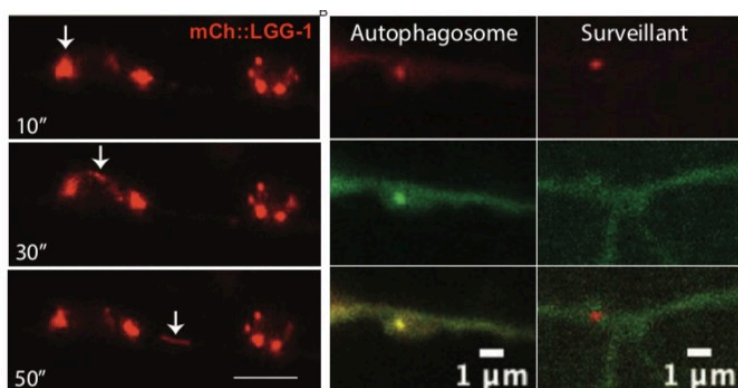
Figure 4: (A) mCherry::LGG-1 surveillants are seen budding off from a cell body. Numbers on bottom left represent time in seconds. **(B)** Image of an autophagosome in the dorsal nerve cord and a surveillant. Autophagosomes are seen labeled with GFP while surveillants are labeled by mCherry. Figure courtesy of Christine Wnukowski.

Typically, synaptic proteins at axons and presynaptic terminals undergo retrograde transport for degradation by somatic lysosomes (Cheng, 2015; Okerlund, 2017), which typically remain constrained to the AIS. Uniquely, surveillants are seen leaving the AIS and travelling to the dorsal cord where the synapses are located (Figure 5, Figure 6). The organelle is seen to have bidirectional movement, undergoing both anterograde and retrograde movement. Surveillants pause at synapses to "surveil" the area and take up synaptic cargo (Wnukowski, 2023). The organelle has been detected taking up α- synuclein which accumulates at neurons in Parkinson's.
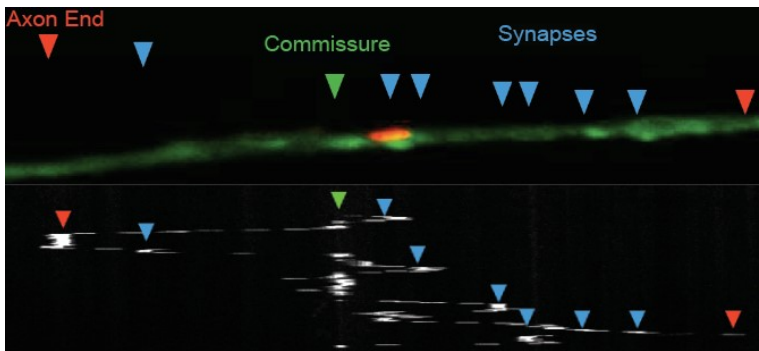
Figure 5: The kymograph confirms the movements of surveillants that have been seen. After the surveillant exited the AIS it visits the dorsal cord and moves back and forth, spending some time there. Figure courtesy of Christine Wnukowski.

Synapses

Dorsal nerve cord
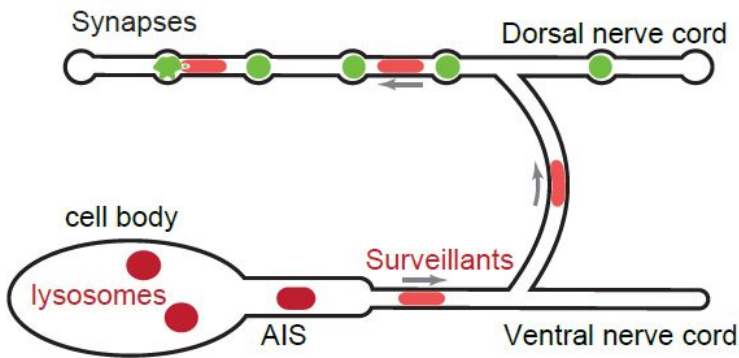
Figure 6: Depiction of the unique movement seen by surveillants (light red) going past the AIS, up to the dorsal cord and synapses. As the surveillants move to the synapse they may be picking up waste or cargo (green) to degrade.

The molecular makeup and cargo that is picked up by

surveillants remains unknown. Therefore, I investigated the co-localization of unique organelle markers with surveillants to understand their identity and origin.

METHODS TABLE 1: STRAINS

Name	Strain	Proteins Colocalized
Wild-type	N2	wild-type
EG10058	LGG-1(oxSi1131[Punc-47::mCh::gfp::lgg-1::let-858]) IV	LGG-1 Tandem Fluorescent
EG10057	oxSi784[Punc-47::GFP::snap-29::let-858utr, CBunc-119+] II oxSi1110[Punc-47::mCh::lgg-1::let-858, CB-unc-119(+)]	SNAP-29/ LGG-1
	5605(oxSi1097[PSnt-1::2xnls-FLP(G5D) unc-119+]) II	
UNC-32	ox904[unc-32::flpon-gfp] III	UNC-32/ LGG-1
	oxSi1110[Punc-47::mCh::lgg-1::let-858, CB-unc-119(+)] IV	
	5605(oxSi1097[PSnt-1::2xnls-FLP(G5D) unc-119+]) II	
RAB-7	rab-7(ox984)[Skylan-S::FLP::rab-7] II	RAB-7/ LGG-1
	oxSi1110[Punc-47:mCherry::lgg-1::let-858, CB-unc-119(+)] IV	
	ox980[cup-15::FLP-on::eGFP] II	
CUP-15	oxEx2241[Psnt1:2xNLS-FLP(G5D), Psnt-1:mTagBFP2, Pmyo2:eGFP, unc-119(+)]	CUP-15/ LGG-1
	oxSi1110[Punc-47:mCherry::lgg-1::let-858, CB-unc-119(+)] IV	

Table 1: List of *C. elegan* strains used throughout the experiment. RAB-7 and CUP-15 have preliminary data on them which is further addressed in the Discussion section.

WORM CARE

Each strain was maintained by transferring worms to new plates twice a week. The plates contained food made up of the *E. coli* strain, OP50. This food source is known as an uracil auxotroph with limited growth on Nematode Growth Medium (NGM). The plates used OP50 and spread over NGM plates.

When transferring the worms to a new plate, a worm picker was used. This consists of a 1 inch 32-gauge platinum wire mounted onto a Pasteur pipet. The wire is used to pick the worms under a microscope by swiping the side of the worm and can be sanitized between transfers by being flamed. Lastly, the worms were consistently stored at 15℃.

MOUNTING & HEATSHOCK

To stage the worms as young adults, L4 worms were picked onto a fresh plate the day before imaging. Worms were heat shocked for 3 hours in a 34℃ incubator, or kept at 15℃ as a control. Worms were then allowed to rest at room temperature for 15 minutes after heat shock, followed by mounting and imaging.

Worms were placed on a 2% agarose, pad mounted on a glass slide into 2 µl of 180 mM muscimol and 2 µl of 30 µm polystyrene beads. A #1.5 coverslip was placed on top and sealed with petroleum jelly to prevent dehydration. The agarose allowed for the worms to stick onto the slide and the muscimol was used to inhibit muscle contractions through interactions with the GABAA receptor UNC-49 (Han, 2015). As a result of this the worm relaxes and becomes immobile. The polystyrene beads provide additional movement restriction

by allowing contact with the glass (worms are ~ 50 µm in diameter) while preventing the worms from being fully compressed.

IMAGING PROCESS

  CONFOCAL AND AIRYSCAN MICROSCOPY

  Confocal and Airyscan imaging was performed on a Zeiss 880 Line Scanning Microscope. Zeiss software was used to capture optical sections in the Z direction. Images were obtained using a Plan-Apochromat 63x/1.4 NA Oil immersion objective. Standard laser lines and filter sets were used to capture mCherry and GFP signals.

  Skylan-S images were collected using GFP lasers and filters along with 0.5% illumination using a 405 nm laser to switch it to the "on" state. Surveillants were identified by scanning the dorsal nerve cord and commissures for Punc-47::mCherry::lgg-1 positive structures.

  IMAGEJ AND COLOCALIZATION SCORING

  Colocalization was manually calculated in FIJI/ImageJ. Areas with a surveillant were deemed a region of interest (ROI) based on fluorescence intensity. The ROI was then used to measure fluorescence intensity of the second fluorescent protein. If the ROI was twice as bright as the background intensity, it was considered colocalized.

RESULTS Using a tandem fluorescent marker (Punc-47::mCherry::GFP::LGG-1), I found that only mCherry fluorescence labels surveillants. mCherry and GFP co-localization would indicate an autophagosome due to the neutral pH of the organelle. Overall, this suggests that surveillants are distinct from autolysosomes, because the GFP signal is quenched (Figure 7). These data suggest that

surveillants might be acidified, although an alternative is that the LGG-1 has passed through a degradative organelle and GFP was degraded.
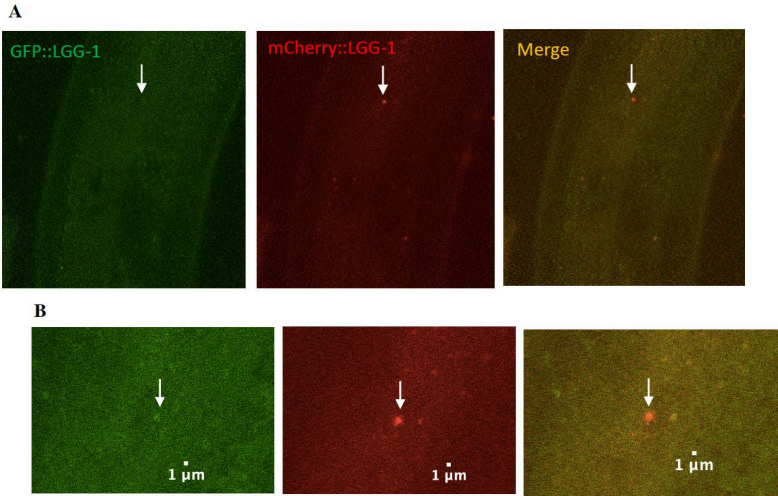
Figure 7: A surveillant seen moving through the commissures. **(A)** The top three images depict surveillants not labeled in the green channel but in the red channel. This indicates that surveillants are not labeled by the autolysosome marker, GFP and is labeled by mCherry. **(B)** The lower three images are a zoomed in look at the images above.

COLOCALIZATION DATA UNC-32

UNC-32 is an ortholog of the a subunit of the V0 sector of the V-ATPase and is highly

expressed in *C.* elegans neurons. UNC-32 is required for acidifying organelles and is present on both lysosomes and synaptic vesicles. UNC-32 was found on 2/11 (18%) of surveillants, suggesting that surveillants do not require UNC-32 (Table 2), (Figure 8, Figure 9). This observation was important in distinguishing the makeup of surveillants from

proteins that are commonly found to be associated with lysosomes.

A
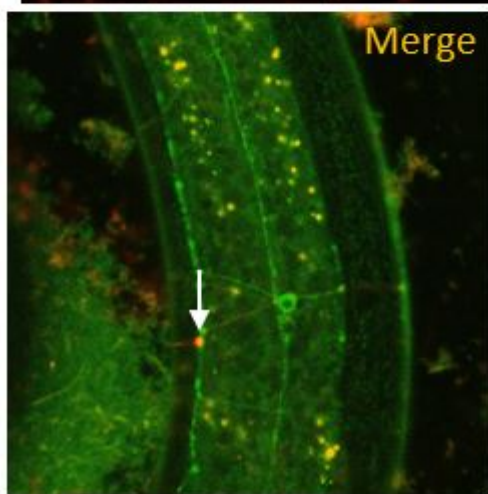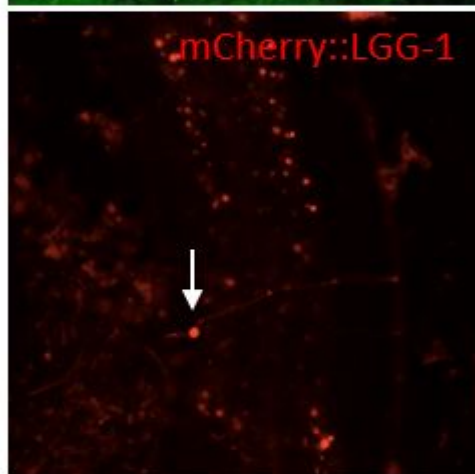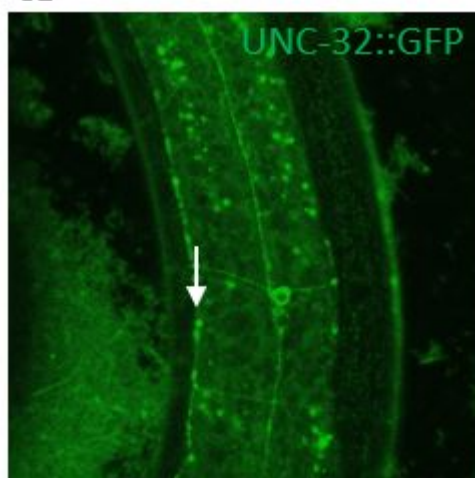
B
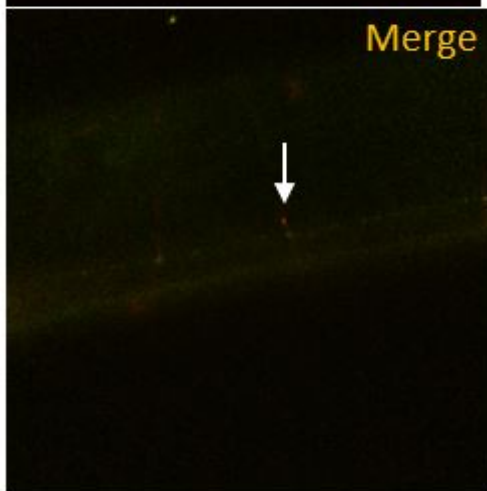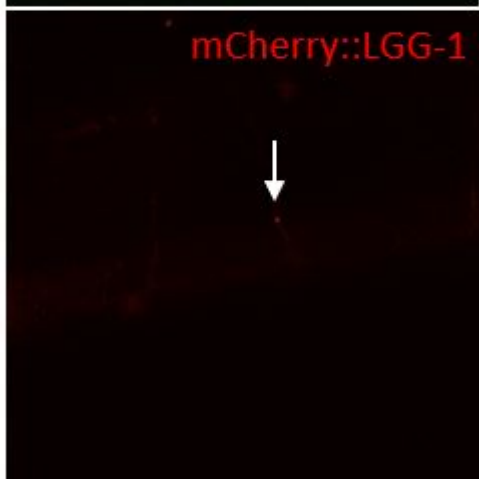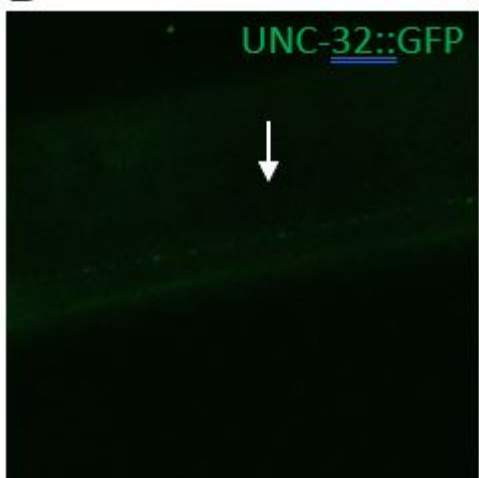
Figure 8: (A) The images in the left column depict what a clear colocalization of UNC-32 and a surveillant looks like. The surveillant was seen travelling down the commissure of the worm.

(B) The images in the right column are images of a surveillant that did not colocalize with UNC-

32. The surveillant was seen travelling down a commissure but is not associated with anything seen in the green channel.

A
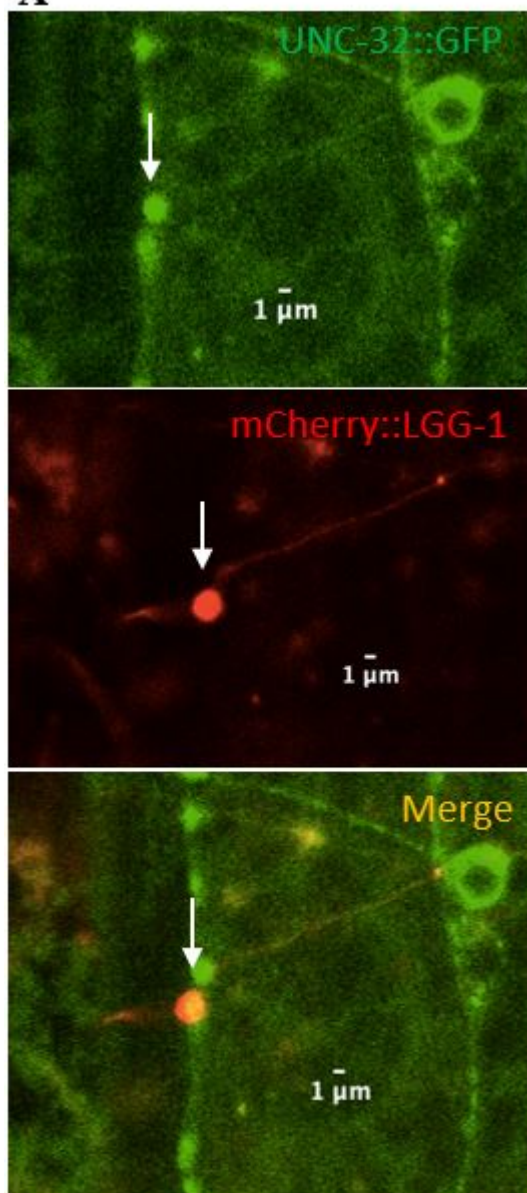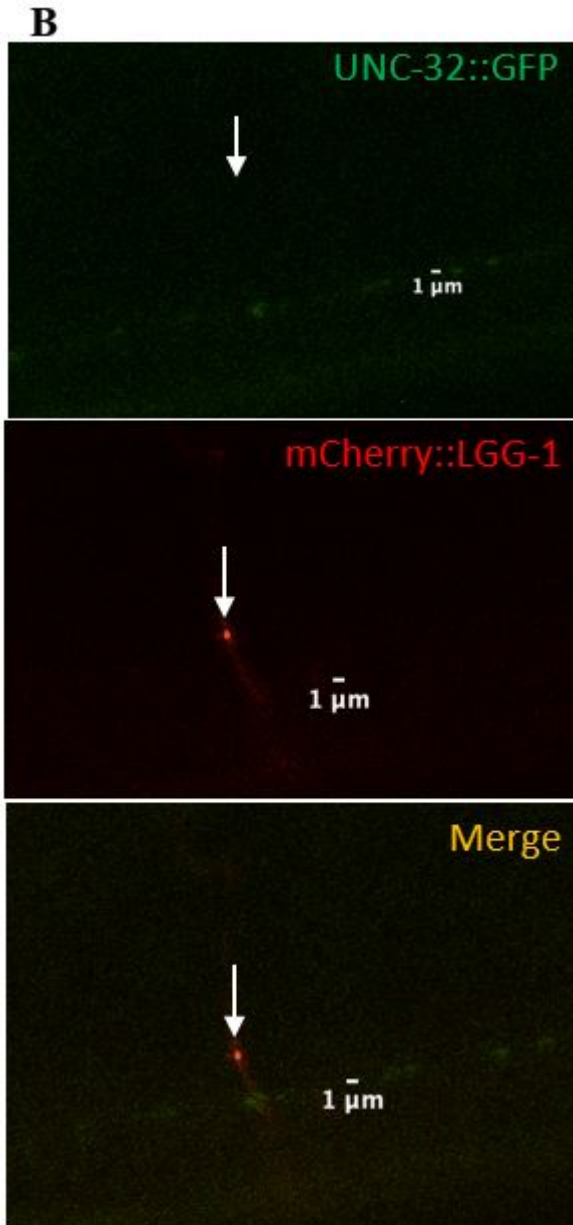
Figure 9: (A) Zoomed in version of the colocalized surveillant shown in Figure 8, column A **(B)** Zoomed in version

of the surveillant that did not colocalize with UNC-32 from Figure 8, column B.

SNAP-29

   SNAP-29 is a member of a SNARE complex, and helps fuse autophagosomes to lysosomes, forming an autolysosome. SNAP-29 colocalizes with mCherry puncta in 14/22 (64%) surveillants. This indicates that surveillants are similar to or may come from autolysosomes (Figure 10, Figure 11).
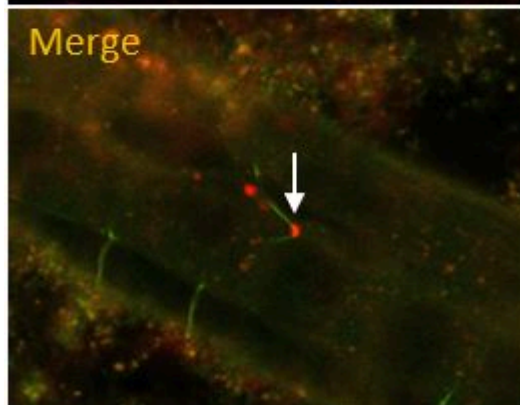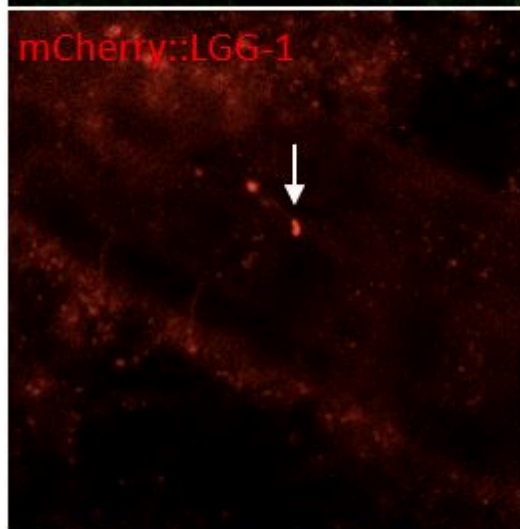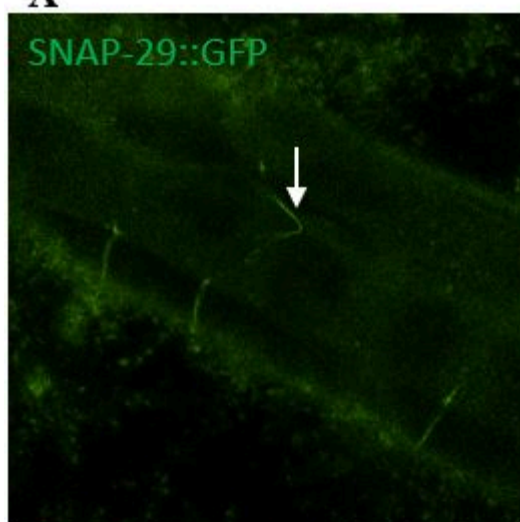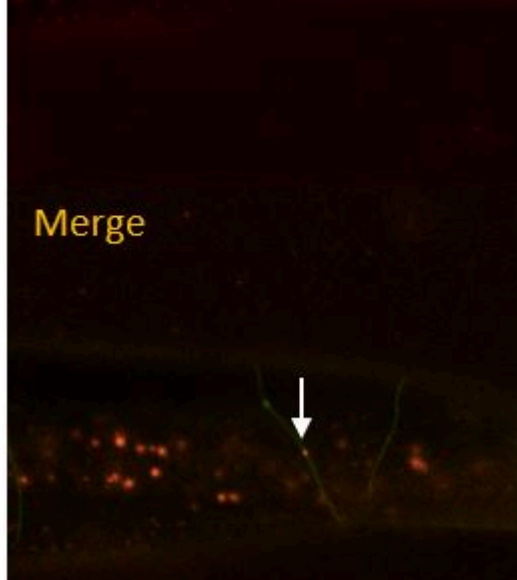
Figure 10: (A) The images on the left depict a surveillant that colocalized with SNAP-29 within a commissure. **(B)** The images in the column on the right depicts a surveillant also seen within a commissure but did not colocalize with SNAP-29.

A
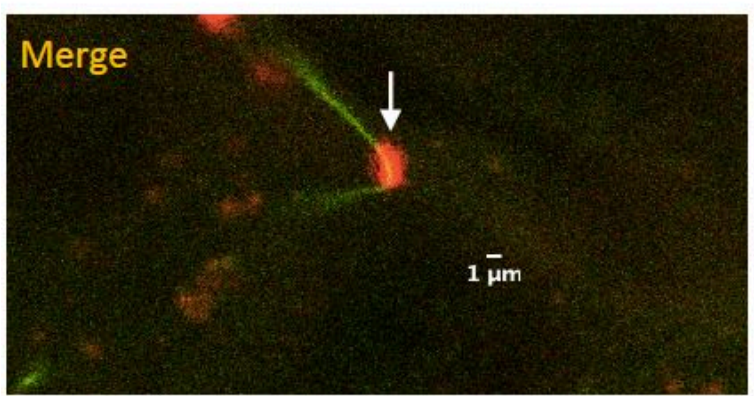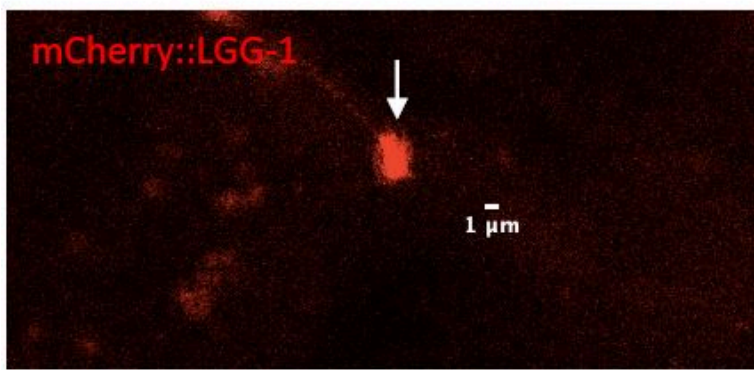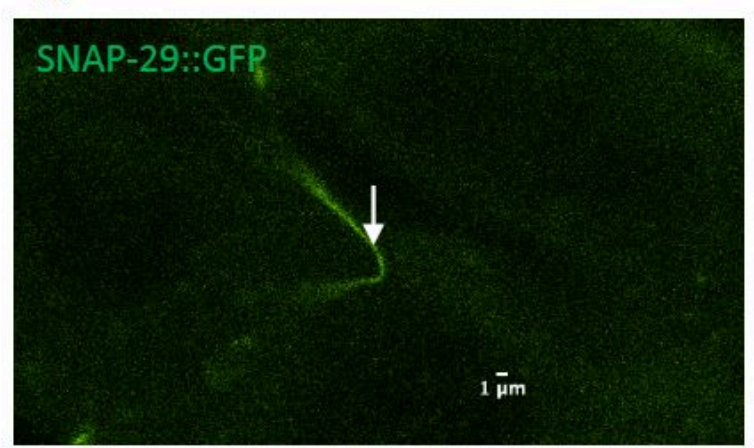
B
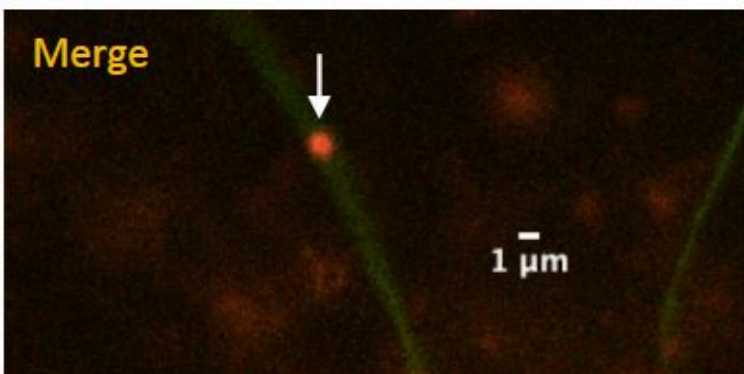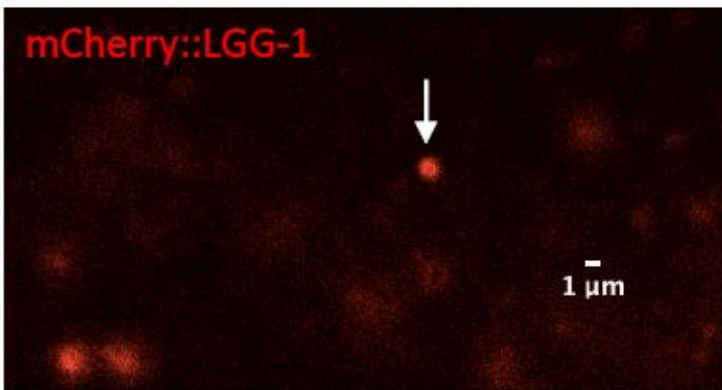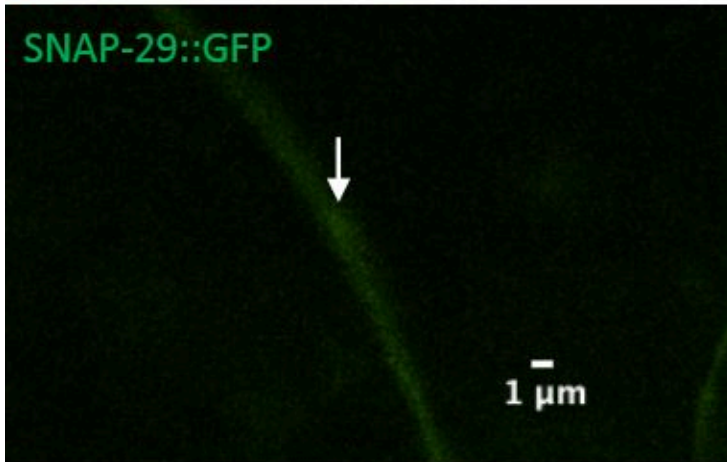
Figure 11: (A) Zoomed in version of the colocalized surveillant shown in Figure 9, column A

(B) Zoomed in version of the surveillant that did not colocalize with SNAP-29 from Figure 9, column B.

TABLE 2: COLOCALIZATION RESULTS

Protein	Amount Colocalized	Amount not Colocalized	Total number of Surveillants Identified	Percent Colocalized
GFP::LGG-1/ mCherry::LGG-1	0	17	17	0%
UNC-32/LGG-1	2	9	11	18%
SNAP-29/LGG-1	14	8	22	64%

DISCUSSIONDisruptions in degradative pathways and accumulation of aggregated proteins are often hallmarks of common neurodegenerative diseases such as Parkinson's disease. How these damaged and aggregated proteins are removed from synapses is still unclear. We have observed that surveillants are likely derived from autolysosomes and may be a unique pathway by which neurons respond to stress.

Surveillants have been detected at synapses using the mCherry::LGG-1 marker. This also indicates that surveillants are distinct from autophagosomes and autolysosomes due to the quenching of eGFP::LGG-1, suggesting an acidic environment.

LIMITATIONS

The heat shock process used in this experiment is essential in detecting surveillants due to their scarcity under basal

conditions. Although the heat shock acting as a stressor to the worms may be a large contributor to the visibility of surveillants, mCherry could potentially contribute as lysosomal stressor (Costantini, 2015; Landgraf, 2013). mCherry may be accumulating in lysosomes because it is resistant to lysosomal proteases (Melentijevic, 2017; Shemiakina, 2012). Additionally, eGFP variants are quenched in acidic environments, making it difficult to identity what proteins are colocalizing with surveillants. Difficulties in labeling lysosomes was another limitation within this experiment. Due to the ambiguous definition of a lysosome there are several distinct markers that can be used. We chose LC3 / LGG-1 since it is also known as an autophagosome marker and allows us to better differentiate and understand the composition of surveillants from other degradative organelles.

FUTURE DIRECTIONS

We currently see that surveillants will take up alpha-synuclein, a protein that is mutated and aggregates in Parkinson's disease. I have also seen that surveillants have colocalized with the proteins RAB-7 and CUP-15. In the future, I would like to look at surveillant co- localization with RAB-7 (a late endosome/lysosome marker), and the lysosome chloride transporter osteoporosis-associated transmembrane protein 1 OSTM1/CUP-15 (a true lysosomal marker). First, RAB-7 is of interest because while the Jorgensen lab has identified that surveillants are not lysosomes, endosomes have the ability to be transported into the axon beyond the AIS, like surveillants. Second, CUP-15 is a specific marker of lysosomes because it has no known association with other organelles. Both genes have been tagged with fluorescent proteins using CRISPR/Cas9. Using the FRT/Flp system, we can induce expression of these

fluorescent proteins specifically in neurons. Another possible protein found in surveillants is the trans Golgi network protein TGN-38. SNAP-29 is associated with TGN-38 that acts as a trans-Golgi shuttle protein. Currently, TGN-38 has been seen to be present on all surveillants. However, when this experiment was done, the protein was overexpressed using an array. To validate colocalization of TGN-38 and surveillants a CRISPR that endogenously tags the protein will have to be generated. This preliminary data suggests surveillants are lysosome-derived organelles that could be transporting or degrading synaptic cargo. The research on surveillants has been important in broadening our understanding of how waste could be removed from neurons to prevent neurodegenerative diseases and how human neuron's respond to severe stress.

REFERENCES1.   Alzheimer's Association. 2023 Alzheimer's Disease Facts and Figures. Alzheimers Dement 2023;19(4). DOI 10.1002/alz.13016.

2.   Bucci, C., Thomsen, P., Nicoziani, P., Mccarthy, J., & Deurs, B. Van. (2000). Rab7: a key to lysosome biogenesis. Molecular Biology of the Cell, 11(February), 467–480.

3.   Cheng, X. T., Zhou, B., Lin, M. Y., Cai, Q., & Sheng, Z. H. (2015). Axonal Autophagosomes recruit dynein for retrograde transport through fusion with late endosomes. Journal of Cell Biology, 209(3), 377–386.

4.   Cheng, Xiu-Tang & Xie, Yuxiang & Zhou, Bing & Huang, Ning & Farfel- Becker, Tamar & Sheng, Zu-Hang. (2018). Characterization of LAMP1-labeled nondegradative

lysosomal and endocytic compartments in neurons. The Journal of Cell Biology. 217. jcb.201711083. 10.1083/jcb.201711083.

5.   Costantini, L. M., Baloban, M., Markwardt, M. L., Rizzo, M., Guo, F., Verkhusha, V. V., & Snapp, E. L. (2015). A palette of fluorescent proteins optimized for diverse cellular environments.   Nature   Communications,   6(May). https://doi.org/10.1038/ncomms8670

6.   Di Maio, Roberto & Barrett, Paul & Hoffman, Eric & Barrett, Caitlyn & Zharikov, Alevtina & Borah, Anupom & Hu, Xiaoping & McCoy, Jennifer & Chu, Charleen & Burton, Edward & Hastings, Teresa & Greenamyre, J.. (2016). – Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinsons disease. Science Translational Medicine. 8. 342ra78-342ra78. 10.1126/scitranslmed.aaf3634.

7.   Edwards, S. L., Yu, S. C., Hoover, C. M., Phillips, B. C., Richmond, J. E., & Miller, K. G. (2013). An organelle gatekeeper function for caenorhabditis elegans UNC-16 (JIP3) at the axon initial segment. Genetics, 194(1), 143–161. https://doi.org/10.1534/genetics.112.147348

8.   Ferguson, S. M. (2017a). Neuronal lysosomes. Neuroscience Letters, 697(October 2017), 1–9. https://doi.org/10.1016/j.neulet.2018.04.005

9.   Gheith, Osama & Wafa, Ehab & Refaie, Ayman & Hassan, Nabil & Ismail, Amani & Sheashaa, Hussein & Shokeir, Ahmaed & Kamal, Mohamed & Ghoneim, Mohamed. (2009). Post-transplant anemia in pediatric patients and its impact on patient and graft survival: single center experience. African Journal of Nephrology. 13. 31-38. 10.21804/13-1-777.

10. Han B, Bellemer A, Koelle MR. An evolutionarily conserved switch in response to GABA affects development and behavior

of the locomotor circuit of Caenorhabditis elegans. Genetics. 2015 Apr;199(4):1159-72. doi: 10.1534/genetics.114.173963. Epub 2015 Feb 2. PMID: 25644702; PMCID:

PMC4391577.https://doi.org/10.1016/j.neuron.2017.01.026

11.   Kundra R, Dobson CM, Vendruscolo M. A Cell- and Tissue-Specific Weakness of the Protein Homeostasis System Underlies Brain Vulnerability to Protein Aggregation. iScience. 2020 Mar 27;23(3):100934. doi:10.1016/j.isci.2020.100934. Epub 2020 Feb 24. PMID: 32146327; PMCID: PMC7063235.

12.   Landgraf, D., Akumus, B., Chien, P., Baker, T. A., & Paulsson, J. (2013). Segregation of molecules at cell division reveals native protein localization. Nature Methods, 9(5), 480–482. https://doi.org/10.1038/nmeth.1955.Segregation

13.   Liao, Y. C., Fernandopulle, M. S., Wang, G., Choi, H., Hao, L., Drerup, C. M., Patel, R., Qamar, S., Nixon-Abell, J., Shen, Y., Meadows, W., Vendruscolo, M., Knowles, T. P. J., Nelson, M., Czekalska, M. A., Musteikyte, G., Gachechiladze,M. A., Stephens, C. A., Pasolli, H. A., ... Ward, M. E. (2019). RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether. Cell, 179(1), 147-164.e20. https://doi.org/10.1016/j.cell.2019.08.050

14.   Lie, P. P. Y., Yang, D. S., Stavrides, P., Goulbourne, C. N., Zheng, P., Mohan, P. S., Cataldo, A. M., & Nixon, R. A. (2021). Post-Golgi carriers, not lysosomes, confer lysosomal properties to pre-degradative organelles in normal and dystrophic axons. Cell Reports, 35(4), 109034. https://doi.org/10.1016/j.celrep.2021.109034

15.   Lukas A Huber, David Teis, Lysosomal signaling in control of degradation pathways, Current Opinion in Cell Biology, Volume 39, 2016, Pages 8-14, ISSN 0955-0674, https://doi.org/10.1016/j.ceb.2016.01.006.

16.   Melentijevic, I., Toth, M. L., Arnold, M. L., Guasp, R.

J., Harinath, G., Nguyen, K. C., Taub, D., Parker, J. A., Neri, C., Gabel, C. V., Hall, D. H., & Driscoll, M. (2017). C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. Nature, 542(7641), 367–371. https://doi.org/10.1038/nature21362

17. Monaco, Antonio & Fraldi, Alessandro. (2020). Protein Aggregation and Dysfunction of Autophagy-Lysosomal Pathway: A Vicious Cycle in Lysosomal Storage Diseases. Frontiers in Molecular Neuroscience. 13. 10.3389/fnmol.2020.00037.

18. Okerlund, N. D., Schneider, K., Leal-ortiz, S., Montenegro-venegas, C., Kim, S. A., Garner, L. C., Gundelfinger, E. D., Reimer, R. J., & Garner, C. C. (2017).Bassoon controls presynaptic autophagy through Atg5. Neuron, 93(4), 897- 913.e7.

19. Prahlad V, Morimoto RI. Integrating the stress response: lessons for neurodegenerative diseases from C. elegans. Trends Cell Biol. 2009 Feb;19(2):52-61. doi: 10.1016/j.tcb.2008.11.002. Epub 2008 Dec 26. PMID: 19112021; PMCID: PMC4843516.

20. Recasens, Ariadna & Dehay, Benjamin & Bové, Jordi & Carballo-Carbajal, Iria & Dovero, Sandra & Pérez, A & Fernagut, Pierre-Olivier & Blesa, Javier & Parent, Annabelle & Perier, Celine & Farinas, Isabel & Obeso, Jose & Bezard, Erwan & Vila, Miquel. (2014). Lewy body extracts from Parkinson disease brains trigger synuclein pathology and neurodegeneration in mice and monkeys. Annals of neurology. 75. 10.1002/ana.24066.

21. Settembre, C., Fraldi, A., Medina, D. L., & Ballabio, A. (2013). Signals from the lysosome : a control centre for cellular clearance and energy metabolism. Nature Publishing Group, 14(5), 283–296. https://doi.org/10.1038/nrm3565

22. Shemiakina, I. I., Ermakova, G. V., Cranfill, P. J., Baird,

M. A., Evans, R. A., Souslova, E. A., Staroverov, D. B., Gorokhovatsky, A. Y., Putintseva, E. V., Gorodnicheva, T. V., Chepurnykh, T. V., Strukova, L., Lukyanov, S., Zaraisky, A. G., Davidson, M. W., Chudakov, D. M., & Shcherbo, D. (2012). A monomeric red fluorescent protein with low cytotoxicity. Nature Communications, 3, 1–7. https://doi.org/10.1038/ncomms2208

23. Sugi T. Genome Editing in C. elegans and Other Nematode Species. Int J Mol Sci. 2016 Feb 26;17(3):295. doi: 10.3390/ijms17030295. PMID: 26927083; PMCID: PMC4813159.

24. Wilfred Lieberthal, (2008), Macroautophagy: a mechanism for mediating cell death or for promoting cell survival?, Kidney International, Volume 74, Issue 5, Pages 555-557, ISSN 0085-2538, https://doi.org/10.1038/ki.2008.325.

25. Wnukowski, C., (2023). Neuronal lysosomes are dynamically managed in number, size, localization, and mobility in response to stress. [Unpublished chapter of doctoral dissertation]. University of Utah.

26. Yu, L., Chen, Y., & Tooze, S. A. (2018). Autophagy pathway: Cellular and molecular mechanisms. Autophagy, 14(2), 207–215. https://doi.org/10.1080/15548627.2017.1378838
Internet Resources:

1. WormBase
   ◦ – Available from: https://wormbase.org/

2. FP Base
   ◦ – Available from: https://fpbase.org/

3. Wormbook
   ◦ – Available from:
     http://www.wormbook.org/

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96. **Research Reflection by Gareema Dhima**

Gareema Dhiman

Faculty Mentor: Erik Jorgensen (School of Biological Sciences, University of Utah)

My undergraduate research experience was invaluable and allowed me to build the confidence to pursue a career in research. Not only was I able to learn important research skills but I was able to apply what I was learning in my classes to a scientific project.

About the Author

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97. Comparative Transcriptomic Assay of Phellinus Tremulae Isolates to Identify the Genetic Responses of Environmental Stress

Tiffany Do; Bryn Dentinger (School of Biological Sciences); and Keaton Tremble

Faculty Mentor: Bryn Dentinger (School of Biological Sciences, University of Utah)

Saprotrophic fungi are a diverse ecological group that can breakdown organic matter to obtain carbon. Due to their metabolic processes, they play an essential role in nutrient cycling within the microbial soil community. Despite the importance of saprotrophic fungi, little is known about their response to climate change. Mimicking real world situations and using transcriptomic analysis can help us characterize what genetic mechanisms saprotrophic basidiomycetes use to respond to environmental changes. This research utilizes cultures of a common saprotrophic fungus, Phellinus tremulae, which is known to cause white trunk rot and parasitize aspen trees. We have conducted a culture growth assay where isolates are grown under varying conditions that mimic ecological stressors, to induce a stress response in the saprotrophic fungus. We then extracted and sequenced mRNA from the cultures to identify the genes that are differentially expressed in the presence or absence of an ecological stressor. Specifically, we analyzed the impacts of heat stress, high and low pH, and recalcitrant carbon compounds as the sole carbon source. Differential gene expression analysis of these conditions revealed that environmental stress, particularly living stress, had a greater impact on isolates compared to nutritional stress. Future work includes identifying if these fungi utilize these genes to adapt to environmental stress in their natural habitats and what cellular functions are involved with adapting to these stressors.

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98. **Research Reflection by Tiffany Do**

Tiffany Do

Faculty Mentor: Bryn Dentinger (School of Biological Sciences, University of Utah)

I've gained various skills including experience in subculturing, creating growth experiments, analyzing growth and population rates, performing RNA extractions, and differential expression analysis. I've also gained valuable field experience from working in a lab as an undergraduate. Being in a lab has allowed me to visualize topics in my courses and gain the necessary experience for future goals and careers.

About the Author

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99.   # Exploring the Impact of Medicines on DNA Repair Enzymes

Mary Fairbanks and Martin Horvath (School Of Biological Sciences)

Faculty Mentor: Martin Horvath (School of Biological Sciences, University of Utah)

Abstract

Medicines are known to alter the gut's microbiome composition and function, but their ability to also impact DNA repair enzymes has not yet been investigated. In this study, we added medicines into bacterial systems to see how mutation rate and DNA repair enzymes were impacted. We found that aspirin was toxic to bacteria and determined a tolerable dose through bacterial growth rate tests. A concentration of 0.01 mg/mL of aspirin showed the same replication rate as when no

medicine was added, so we kept the dose below this threshold to maintain a level playing field for the following mutation suppression assay. The mutation frequency rates of bacteria exposed with various concentrations of medicines were measured through counting rifampicin resistant mutants that spontaneously arose in an overnight culture. The median mutation rate for aspirin was 22 per 100 million cells plated (14-29, 95% confidence interval; CI), which was not significantly different from the rate measured for water at 23 (19-28, 95% CI). Similarly, the median mutation rate for adapalene was 19.5 (16-28, 95% CI), which was not significantly different from the rate measured for DMSO at 24.5 (21-33, 95% CI). These results show that aspirin and adapalene do not impact mutation rates in our laboratory strain of bacteria. Previous studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) increase the likelihood of infection from *Clostridioides difficile* by diminishing microbiome diversity and resistance, which raises concerns about medicines negatively impacting the microbiome. Our results reassure us that medicines like aspirin and adapalene probably do not have off-target impacts on DNA repair enzymes, and thus are unlikely to disrupt the mutation rate of bacteria in our microbiome.

Introduction

Commonly used medicines treat specific symptoms or illnesses but may have unintended side effects. Aspirin is taken to reduce pain and fever but is sometimes used to prevent heart attacks and ischemic strokes (strokes that occur when the flow of blood to the brain is blocked) (Brazier 2020). Aspirin belongs to a group of medicines called salicylates, which stop

the production of certain natural substances that cause fever, pain, swelling, and blood clots. A rare side effect of daily low-dose aspirin is hemorrhagic stroke (Brazier 2020). Other side effects of aspirin include irritation of the stomach lining and digestive problems (Zimlich 2022). Variations in drug outcomes encourage us to investigate pathways at a molecular level in order to improve drug-targeting efficiency, reduce side effects, and uncover hidden uses. Drug disposition (absorption, distribution, metabolism, and excretion) and pharmacokinetics (the rate of these processes and concentration compared to time) play a vital role in drug efficacy and outcome (Caldwell, Gardner, and Swales 1995). Thus, drug interactions should be investigated in different environments in our body. Experimenting with medicines' impact on bacteria is particularly important to depict environments like our microbiota with a non-invasive model system.

The relationship between medicines and bacteria in the gut microbiome is complex and bi-directional (Reviewed in Weersma, Zhernakova, and Fu 2020). Changes in the gut microbiome composition can be influenced by drugs, and likewise, the gut microbiome can influence drug activity by altering bioavailability of the drug. Gut microbes can impact the efficiency of a drug by transforming its structure which may change drug bioavailability, activity, or toxicity, now referred to as pharmacomicrobiomics (Koppel, Rekdal, and Balskus 2018). For example, the oral antiviral drug brivudine can be metabolized to bromovinyluracil by five major phyla that dominate the mammalian gut microbiota (Reviewed in Zimmermann et al. 2019).

Microbiome composition influences drugs and vice versa. Recent findings show commonly used non-antibiotic drugs such as proton pump

inhibitors (PPIs) and metformin change the microbiome composition, biodiversity, and function (Freedberg et al. 2015). Additionally, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with enhanced susceptibility to *Clostridioides difficile* infection (CDI) with increased severity by decreasing colonization resistance and biodiversity (Bonder et al. 2016). CDI is a major public health threat that can range from diarrhea to life threatening damage to the colon. NSAIDs inhibit the activity of cyclooxygenase (COX) and lead to the formation of prostaglandins (PG), which alter the inflammatory response pathway. In a recent study, the NSAID indomethacin was found to dramatically increase CDI severity through inhibition of intestinal tissue immune response (Maseda et al. 2019). Indomethacin inhibited PG biosynthesis, which increased intestinal tissue histopathology due to dismantled intestinal epithelial tight junctions allowing partial bacterial diffusion (Maseda et al. 2019). The microbiome's composition being impacted by medicine and its ability to respond to that medicine shows an example of the interaction between drugs and gut microbiome to be both complicated and interconnected.

Moreover, medicines intended to treat cancer may have off-target molecular interactions that affect DNA repair function. Chronic intake of the NSAID aspirin was shown to reduce the risk of

colorectal cancer (Gwyn and Sinicrope 2002). The GO DNA repair pathway is known to be involved in cancer reduction (Curtin 2012). A member of the GO DNA repair pathway, the DNA glycosylase MUTY, initiates the repair process at lesions where an oxidized purine 8-oxo-7, 8-dihydroguanine (8-oxoG) is mispaired with an adenine (A). DNA repair enzymes are responsible for preventing mutations that lead to cancerous growth in humans, so aspirin may reduce the risk of colon cancer by impacting the function of DNA repair enzymes. Variants of the mammalian homolog MUTYH are known to increase the risk of cancer (Picanço-Albuquerque et al. 2022). To explore the idea that aspirin and other medicines can interact with MUTYH, medicines were docked to MUTYH protein with favorable binding energies (Sehgal 2021). Additionally, MutY glycosylase assays performed by Harini Srinivasan and Sonia Sehgal found adapalene to reduce the activity of bacterial MutY (Srinivasan 2022). The finding that adapalene inhibits MutY suggests that it may also inhibit human MUTYH which could be linked to a higher risk for cancer.

To guide our research, we posed the following questions: do these medicines have the potential for treating or preventing colorectal cancer because they act on DNA repair enzymes? Are the DNA repair enzymes in our cells and our gut microbiota being impacted when we take these medicines? To

answer these questions, we added medicines into bacterial systems to see how bacterial mutation rate and DNA repair enzymes were affected (Figure 1). Mutations rates were measured in the presence of medicines to evaluate MutY function. Since mutation rate and replication rate are related, replication rate with and without the medicine was also measured to set a baseline for the mutation rate assay. Noticeable differences in bacterial growth rates were observed when adding medicines like aspirin, but are unlikely due to impacting the function of DNA repair enzymes like MutY as no impact on the mutation rate was detected.

Figure 1. Concept map showing the relationship between medicines, MutY, and cancer and bacterial mutation.
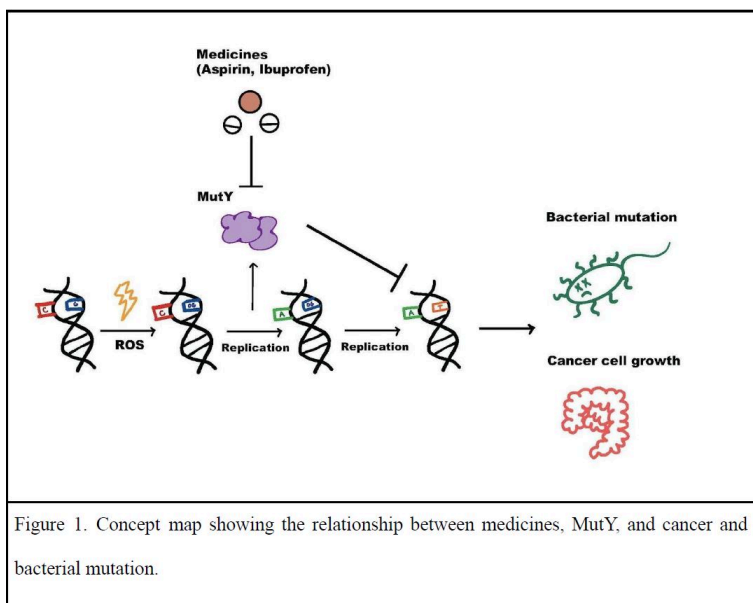
Results

The ability for FDA approved drugs to bind to MutY was first visualized using a virtual modeling program to calculate favorable binding energies within the protein substrate interactions. Medicines were narrowed down through a screening program using AutoDock Vina, which calculates favorable binding affinities. Aspirin and ibuprofen were docked to a bacterial MutY structure and shown to have favorable binding sites (Figures 2 and 3). Aspirin, ibuprofen, adapalene, and other similar structures were also shown to favorably bind to sites in the amino terminal domain (NTD) and carboxyterminal domain (CTD) of MutY.
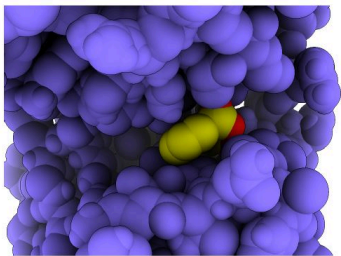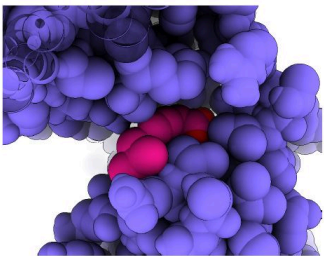
Figure 2. Aspirin (yellow) docked with MutY (blue)	Figure 3. Ibuprofen (magenta) docked with MutY (blue)

Figure 2. Aspirin (yellow) docked with MutY (blue)

Figure 3. Ibuprofen (magenta) docked with MutY (blue)

Bacteria expressing MutY were grown in the presence of these medicines. Initial trials tested 10mg/mL of aspirin added to 2mL overnight culture of KAT media. Culture tubes with aspirin had no growth compared to those that had no medicine. Medicines were then spotted to media plates using a Kirby Beuer technique, this time including ibuprofen. There was a clear zone of bacterial inhibition when aspirin was added to the plates as opposed to ibuprofen which had no zone of inhibition (Figure 4). Toxicity of aspirin was then examined by viewing relative sizes of zones of inhibition in various dilutions of medicine to verify a safe, non-toxic level (Figure 4). Since MutY works on DNA damage before replication, the growth rate of bacteria with various concentrations of medicine was measured to accurately represent mutation occurrences in the proceeding assay. A replication rate across the tested types of medicine was also needed to create an equal level playing ground through the adjustment of concentrations. Growth of bacteria populations was monitored by optical density over time. This growth test was repeated multiple times to plot 2-3 trials for each concentration of aspirin in order to examine which concentration had similar replication rates to the control (no medicine) (Figure 5). Aspirin was found to be tolerated at a low concentration of 0.01mg/mL while ibuprofen, DMSO, and water had no impact on growth rate.
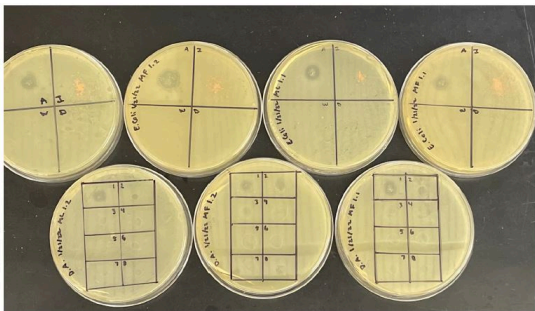
Figure 4. Kirby-Bauer test for aspirin (A), ibuprofen (I), water (W), and DMSO (D). Bottom: Dilution panel for aspirin.
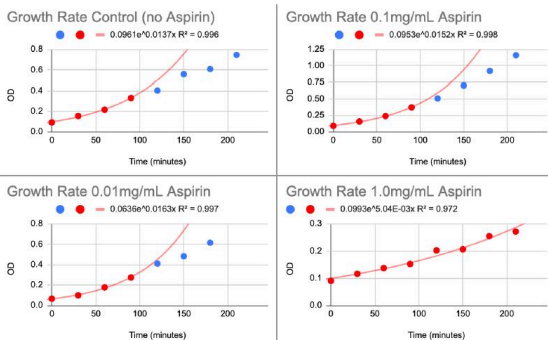
Figure 5. Optical density of bacterial growth over time to measure replication rate constant in different concentrations of aspirin.

To measure mutation rates, several replica cultures were grown with and without medicine by students in the Molecular Biology of DNA Lab course (BIOL 3510, Fall 2022). Spontaneous mutations arising in these cultures were detected by acquired ability to grow on plates containing the antibiotic rifampicin. The boxplot created by the data from members of the DNA Lab Fall 2022 showed a far higher mutation frequency for cultures that do not express MutY (null) compared to cultures that

express a functional MutY (Figure 6). This pattern indicates that the mutation rate assay is working as intended, as a higher mutation rate is expected if DNA repair enzymes are missing or not functioning properly. The mutation frequency measured for cultures expressing MutY and grown in the presence of medicine was highly comparable to that measured in the absence of added medicines (Figure 6).
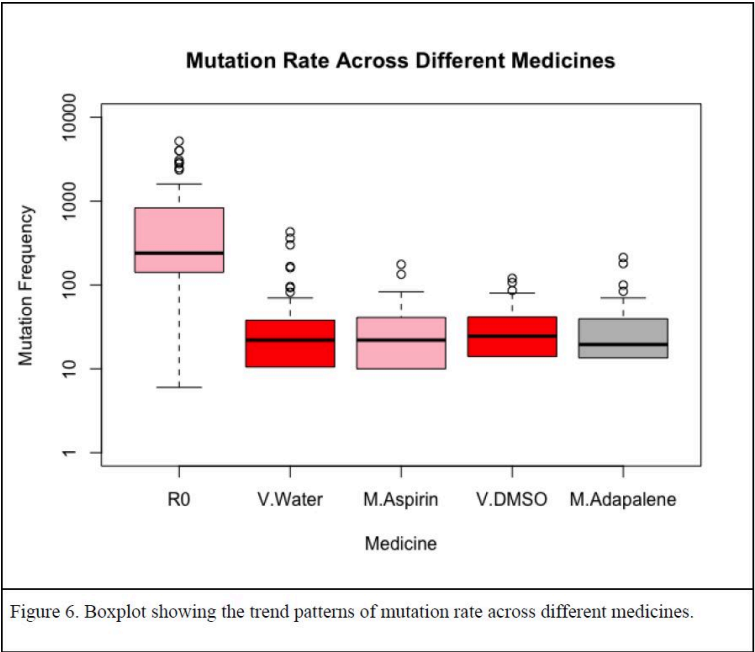
Figure 6. Boxplot showing the trend patterns of mutation rate across different medicines.

The statistical significance of these comparisons was evaluated with a bootstrap method to provide confidence intervals. Overlapping confidence intervals indicate the data are highly comparable, whereas non-overlapping confidence intervals indicate the data are noticeably different at the 95% confidence level ($p < 0.05$). As reported in Table 1, null and MutY

expressing cultures differ significantly in the measured mutation frequency as indicated by non-overlapping confidence intervals. By contrast, adding medicine to the cultures did not impact mutation rates as indicated by overlapping confidence intervals for control cultures grown with added vehicles and cultures grown in the presence of medicines (Table 1).

Table 1. Median Mutation Frequencies with Confidence Intervals*			
Culture	Median	95% C.I. Low	95% C.I. High
Null	240	180	500
V. Water	23	19	28
M. Aspirin	22	14	29
V. DMSO	24.5	21	33
M. Adapalene	19.5	16	28

*Confidence intervals were calculated by a bootstrap method and show statistical significance by nonoverlap.

Discussion

Understanding how medicines impact the function of MutY will not only be applicable to knowing its impact on cancer, but also the mutation rate of enzymes in our microbiota. Learning how these medicines impact bacterial enzymatic function will teach us ways to increase drug efficiency and improve various aspects of our health. Biological tests were put on hold as COVID 19 shut down in person lab work. We stepped back from lab work to focus on designing experiments with as much information as we could get from online sources. Previous experiments conducted by Sonia Seghal and Harini Srinivasan (former Horvath lab members) showed the glycosylase activity

of MutY to be influenced by medicines, especially adapalene which normally treats acne. These findings encouraged us to dock other medicines to DNA repair enzymes with molecular modeling tools/programs to see if binding affinity was favorable. To explore the role that medicines have on DNA repair enzymes, medicines were docked to a crystal structure of MutY using a virtual protein modeling software program and docked using AutoDock VINA. This program calculated favorable binding energies within MutY and its OG:A substrates (oxidized guanine:adenine). Aspirin, ibuprofen, adapalene, and other medicines were also shown to favorably bind to sites in the N terminal domain where adenine interacts with catalytic residues and in the C terminal domain which recognizes the damaged base OG. These favorable binding interactions added support to the idea that medicines have the potential to alter the function of DNA repair enzymes. When we were able to return to lab work, we measured mutation rates by counting mutants that spontaneously arose in overnight cultures of bacteria exposed with various concentrations of medicines. To our surprise, adding aspirin killed overnight cultures. Toxicity to aspirin was a major impediment to testing that idea that aspirin impacts DNA repair in living cells as dead cells cannot mutate. We reaffirmed the concentrations medicines have an effect on growth or replication rate through toxicity tests and bacterial population growth curves. A tolerable dose was determined through comparing growth rates of bacteria cultures in media with varying concentrations of aspirin in comparison to control cultures which were grown in media with no added aspirin. A concentration of 0.01 mg/

mL of aspirin showed the same replication rate as when no medicine was added, so we kept the dose below this threshold to maintain a level playing field between the tested medicines. With concentrations adjusted, the median mutation rate for aspirin and adapalene was found to not significantly differ from the solvents water and DMSO. These results show that aspirin and adapalene do not impact mutation rates in our laboratory strain of bacteria. Previous studies suggest that NSAIDs like aspirin increase the likelihood of infection from *Clostridioides difficile* by diminishing microbiome diversity and resistance, which raises concerns about medicines negatively impacting the microbiome (Maseda et al. 2019). However, our results show that medicines like aspirin and adapalene probably do not have off-target impacts on DNA repair enzymes, and thus are unlikely to disrupt the mutation rate of bacteria in our microbiome.

The molecular modeling and biochemistry results have varied findings on the impact of medicines on DNA repair function. We thought that the medicines may act as competitive inhibitors and therefore reduce enzymatic function. When medicines were docked, we saw binding of both aspirin and ibuprofen to the active site and predicted medicines would impact function. Through findings from a biochemical glycosylase assay, adapalene was seen to reduce the function of

bacterial MutY in an isolated system (only protein and substrate). Reduction in function of the MutY enzyme could be explained by competitive inhibition from adapalene binding to the active site. However, the function of MutY in a biological system appeared to not be impacted as mutation rates measured for bacterial cultures were relatively unchanged by medicines. The differing results seen in a biochemical and biological setting may be due to drug bioavailability, which refers to the extent and rate that a drug is absorbed into a living system. If the medicine cannot get into the bacterial cells or if the medicine is being metabolized by the bacteria then the opportunity for interacting with the DNA repair enzyme would be lost. While the biochemical and biological approaches had varying results, information from both systems can help us uncover mechanisms involving drug pathways.

The tested medicines were not seen to have an impact on bacterial mutation rates through use of DNA repair enzymes like MutY. However, these findings can help fine-tune pathways to increase drug efficiency and explore unknown roles about other similar protein-substrate interactions. For example, a eukaryotic homolog to the enzyme in this experiment is MUTYH, which when dysfunctional may lead to the inheritable MUTYH-associated polyposis (MAP) variants linked to colorectal cancer in humans (Nakamura et al. 2021).

This homolog interests us as we would like to see its impact in medical settings. Understanding the relationship between commonly taken medications and their corresponding enzymatic pathways might inspire ways to improve cancer treatments. Medications that cause inhibition of DNA repair enzymes such as MUTYH may enhance the use of current therapies such as chemotherapy and radiation therapy, which induce DNA damage. Alternatively, increased function in a particular repair pathway could also be used as a monotherapy by selectively killing cancer cells.

Future Directions

Through previous studies of cell survival with some of our tested medicines, aspirin was thought to promote apoptosis through inducing an endoplasmic reticulum (ER) stress response (Ausina et al. 2020). The inhibited growth seen in aspirin could be explained by salicylic acid damaging the cell membrane, but does not account for if salicylic acid induces an ER stress response, as ER is only present in eukaryotes. In future experiments, yeast should be tested to see if aspirin makes a more noticeable difference in cell survival or mutation rates. Switching to a eukaryotic model system will help explain the mechanisms involved in both our cells' and our microbiota's DNA repair enzymes when taking commonly used medications.

Methods

To examine the impact of FDA approved medicines on MutY, mutation rates of bacterial cultures with and without medicines were tested. A plasmid encoding MutY was transformed into the CC104 reporter strain that otherwise does not express MutY or MutM. Null cultures with no functioning MutY and other cultures containing functioning MutY genes were grown overnight. Some of the cultures with MutY were treated with medicine and the other cultures were treated with the vehicle water or dimethyl sulfoxide (DMSO). Culture media additionally contained 10 µg/mL kanamycin, 100 µg/mL ampicillin and 12.5 µg/mL tetracycline. Cultures were grown for 18 hours at 37 degrees with shaking at 180 rpm. Cells were harvested by centrifugation and washed in 0.85% sodium chloride before seeding the equivalent of 100 µL culture on rifampicin plates to measure the frequency of rifampicin resistant spontaneous mutants. Washed cells were additionally diluted 1:107 and seeded on media plates without rifampin to measure cell density. These values were analyzed and plotted with R.

Acknowledgments

ReferencesAusina, Priscila, Jessica R. Branco, Thainá M. Demaria, Amanda M. Esteves, João Gabriel B. Leandro, Alan C. Ochioni, Ana Paula M. Mendonça, et al. 2020. "Acetylsalicylic Acid and Salicylic Acid Present Anticancer Properties against

Melanoma by Promoting Nitric Oxide-Dependent Endoplasmic Reticulum Stress and Apoptosis." *Scientific Reports* 10 (1): 19617. https://doi.org/10.1038/s41598-020-76824-6.

Bonder, Marc Jan, Ettje F. Tigchelaar, Xianghang Cai, Gosia Trynka, Maria C. Cenit, Barbara Hrdlickova, Huanzi Zhong, et al. 2016. "The Influence of a Short-Term Gluten-Free Diet on the Human Gut Microbiome." *Genome Medicine* 8 (April): 45. https://doi.org/10.1186/s13073-016-0295-y.

Brazier, Yvette. 2020. "Aspirin: Health Benefits, Uses, Risks, and Side Effects." September 11, 2020. https://www.medicalnewstoday.com/articles/161255.

Caldwell, J., I. Gardner, and N. Swales. 1995. "An Introduction to Drug Disposition: The Basic Principles of Absorption, Distribution, Metabolism, and Excretion." *Toxicologic Pathology* 23 (2): 102–14. https://doi.org/10.1177/019262339502300202.

Curtin, Nicola J. 2012. "DNA Repair Dysregulation from Cancer Driver to Therapeutic Target." *Nature Reviews Cancer* 12 (12): 801–17. https://doi.org/10.1038/nrc3399.

Freedberg, Daniel E., Nora C. Toussaint, Sway P. Chen, Adam J. Ratner, Susan Whittier, Timothy C. Wang, Harris H. Wang, and Julian A. Abrams. 2015. "Proton Pump Inhibitors Alter Specific Taxa in the Human Gastrointestinal Microbiome: A Crossover Trial." *Gastroenterology* 149 (4): 883-885.e9. https://doi.org/10.1053/j.gastro.2015.06.043.

Gwyn, Karin, and Frank A Sinicrope. 2002. "Chemoprevention of Colorectal Cancer." *The American Journal of Gastroenterology* 97 (1): 13–21. https://doi.org/10.1016/S0002-9270(01)03997-1.

Koppel, Nitzan, Vayu Maini Rekdal, and Emily P. Balskus. 2018. "Chemical Transformation of Xenobiotics by the Human Gut Microbiota." *Science (New York, N.y.)* 356 (6344): eaag2770. https://doi.org/10.1126/science.aag2770.

Maseda, Damian, Joseph P. Zackular, Bruno Trindade, Leslie Kirk, Jennifer Lising Roxas, Lisa

M. Rogers, Mary K. Washington, et al. 2019. "Nonsteroidal Anti-Inflammatory Drugs Alter the Microbiota and Exacerbate Clostridium Difficile Colitis While Dysregulating the Inflammatory Response." *MBio* 10 (1): e02282-18. https://doi.org/10.1128/mBio.02282-18.

Picanço-Albuquerque, Clarissa Gondim, Rosane Oliveira Sant'Ana, Maria Claudia dos Santos Luciano, Maria Júlia Barbosa Bezerra, Flávio da Silveira Bitencourt, Francisca Fernanda Barbosa Oliveira, Thuany Pinto Rocha de Souza, Paulo Goberlânio de Barros Silva, Marcos Venício Alves Lima, and Isabelle Joyce de Lima Silva-Fernandes. 2022. "MUTYH-Related Polyposis Syndrome Associated with a Variant of Uncertain Significance Mutation: A New Pathogenic Mutation?" *Journal of Clinical Oncology* 40 (16_suppl): e22528–e22528. https://doi.org/10.1200/JCO.2022.40.16_suppl.e22528.

Sehgal, Sonia. 2021. "EXPLORING THE ROLE OF BIOLOGICAL PROBES IN DNA REPAIR ENZYME MUTYH." https://doi.org/2021.

Srinivasan, Harini. 2022. "Srinivasan_Thesis.Pdf." https://drive.google.com/file/d/1TvQThafeo_HqHiRkoHruWwBIEeeJwr4h/view?usp=embed_facebook.

UCSF Chimera–a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.

Weersma, Rinse K., Alexandra Zhernakova, and Jingyuan Fu. 2020. "Interaction between Drugs and the Gut

Microbiome." *Gut* 69 (8): 1510–19. https://doi.org/10.1136/gutjnl-2019-320204.

Zimlich, Rachael. 2022. "Aspirin vs. Ibuprofen: How Are They Different, When to Use Each." Healthline. February 2, 2022. https://www.healthline.com/health/aspirin-vs-ibuprofen.

Zimmermann, Michael, Maria Zimmermann-Kogadeeva, Rebekka Wegmann, and Andrew L. Goodman. 2019. "Separating Host and Microbiome Contributions to Drug Pharmacokinetics and Toxicity." *Science (New York, N.Y.)* 363 (6427): eaat9931. https://doi.org/10.1126/science.aat9931.

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100. **Research Reflection by Mary Fairbanks**

Mary Fairbanks

Faculty Mentor: Martin Horvath (School of Biological Sciences, University of Utah)

Working in Dr. Horvath's lab has taught me how to be curious and what it means to be dedicated to a project regardless of the results. I've been encouraged to ask questions and explain findings to form a cohesive pattern that tells a story. Through research and being involved in the DNA lab taught by Dr. Horvath, I have been able to see a project from start to finish. Being able to design my own experiments has given me the opportunity to act as a scientist. I have been able to improve my critical thinking skills and laboratory technique, as well as adapt to change. Having to constantly adjust experiments to fit our specific scenarios taught me to be more flexible. Years of readjustment with varying results taught me persistence. I have grown through research and it continues to

expand my view of the possibilities of innovation. In a career in the medical field, I believe my experience in research will make me a more open-minded thinker, accepting of change.

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101.

Representations of the Symmetric Group from Geometry

Emil Geisler

Faculty Mentor: Sean Howe (Mathematics, University of Utah)

Representation stability was introduced to study mathematical structures which stabilize when viewed from a representation theoretic framework. The instance of representation stability studied in this project is that of ordered complex configuration space, denoted $\mathrm{PConf}_n(\mathbb{C})$:

$$\mathrm{PConf}_n(\mathbb{C}) := \{(x_1, x_2, \ldots, x_n) \in \mathbb{C}^n \mid x_i \neq x_j\}$$

$\mathrm{PConf}_n(\mathbb{C})$ has a natural S_n action by permuting its coordinates which gives the cohomology groups $H^i(\mathrm{PConf}_n(\mathbb{C}); \mathbb{Q})$ the structure of an S_n representation. The cohomology of $\mathrm{PConf}_n(\mathbb{C})$ *stabilizes* as n tends toward infinity when viewed as a family of S_n representations. From previous work, there is an explicit description for $H^i(\mathrm{PConf}_n(\mathbb{C}); \mathbb{Q})$ as a direct sum of induced representations for any i, n, but this description does not explain the behavior of families of irreducible representations as $n \to \infty$. We implement an algorithm which, given a Young Tableau, computes the cohomological degrees where the corresponding family of irreducible representations appears stably as $n \to \infty$. Previously, these values were known for only a few Young Tableaus and cohomological degrees. Using this algorithm, results have been found for all Young Tableau with up to 8 boxes and certain Tableau with more, which has led us to conjectures based on the data collected.

About the Author

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102. **Research Reflection by Emil Geisler**

Emil Geisler

Faculty Mentor: Sean Howe (Mathematics, University of Utah)

In my sophomore year (Fall 2020), I began my first undergraduate research experience under Dr. Ganesh Gopalakrishnan in computer science. The general project goal was to develop efficient neural network compression techniques. Once a neural network is trained, it is typically efficient at computing an output for any given input. However, the limiting constraint in settings like mobile devices tends to be the memory cost of storing the entire neural network architecture. Due to this high memory cost, there is a need to compress the neural network without losing specificity and accuracy. Specifically, my goal was to study and optimize rank selection algorithms for tensor decomposition techniques. This was my first experience with independent college-level academic work. Due to my lack of familiarity with machine

learning and software tools, along with the challenges of the COVID-19 pandemic at the time, this research project was especially challenging for me to produce results. However, I was able to gain knowledge about deep learning by watching a lecture series on modern architectures, including convolutional neural networks and graph neural networks. Furthermore, I learned about tensors in the setting of computer science and optimization and their main methods of compression. Additionally, I developed skills required to read and interpret scientific research papers. As part of my work with Dr. Gopalakrishnan, I read several current research papers and presented findings on novel techniques in model compression to the research group.

In Spring 2021, I began working with Dr. Sean Howe on a mathematics research project in the area representation stability. It has been previously shown that a certain mathematical object (the cohomology of complex configuration space when viewed as a representation of the symmetric group) stabilizes as its degree tends toward infinity, but very little is known about its stable asymptotic structure. My research has been concerned with describing this stable structure. Due to a connection between geometry and arithmetic established by Grothendieck in the 1960's, there is a way to phrase the geometric problem of interest as the weighted average of a family of random variables. Using this framework, it is possible to explicitly solve for the simplest parts of the desired stable structure. To understand the problem, I studied Representation Theory by Fulton and Harris and Algebraic Topology by Hatcher to gain the background needed to understand the definition of the stable structure. I was concurrently enrolled in graduate-level algebra and undergraduate topology courses, which supplemented the

learning required for my research. At first, I was able to write a computer program which determines the stable structure in relation to the exterior powers of the standard representation of the symmetric group. This was the simplest non-trivial computation and a natural starting point. After studying more prerequisite mathematics, I developed an algorithm in Summer 2022 to compute the desired stable structure for any representation of the symmetric group. The results are now solely limited by computational time. I computed coefficients of the first 100 simplest cases, producing novel data which can be used by the mathematical community to better understand the desired stable structure. The field of representation stability is relatively new, having been developed within the last 10 years. Complex configuration space represents the simplest non-trivial example of representation stability. Before the results of my research, it was known that certain properties of this space stabilized as its degree tended toward infinity, but relatively little was known about this stable structure. My novel computational results provide an explicit example of a stable structure which provide a starting point for future theorems. I developed conjectures arising from my algorithmic results and am currently attempting to construct proofs. While the theoretical results are still preliminary, it is exciting to be working on understanding an unsolved math problem. I am currently preparing to submit my results to the Journal of Experimental Mathematics and to undergraduate mathematics journals so other researchers can benefit from my computational results.

In Summer 2021, I attended a summer math research experience for undergraduates (REU) at UC Davis where I worked on a problem in topological data analysis on the evolution of non-segmented RNA based viruses. One of the

primary challenges of treating RNA viruses is their rapid and unpredictable mutations. Non-segmented RNA viruses are subject to random pointwise mutations over generations, but also more complex mutations broadly termed recombination events where entire portions of the RNA sequence are rearranged, replaced, or a combination of both. The standard method for modelling evolution in RNA viruses is phylogenetic trees which is effective for simpler pointwise mutations. However, phylogenetic trees have proven ineffective at modelling recombination events. Persistent homology, a technique from topological data analysis, has previously been proposed as a method to detect recombination events in RNA viruses. During the 8-week program, I studied the previous applications of persistent homology and identified which homological statistics best detect recombination, especially in large datasets.

My undergraduate research experiences have been instrumental in my decision to pursue a Ph.D. In my first years of college, I had still not decided whether to pursue a PhD in computer science or mathematics and sought advice to help inform my decision. For years, I have dreamed of one day pursuing a PhD in pure math and becoming a mathematical researcher. However, I felt discouraged from pursuing this career path because of my perception that math could only be pursued by a limited number of mathematical prodigies and career opportunities were limited. Furthermore, I was concerned that math research was primarily focused on solving Hilbert's 23 problems. As I talked with pure mathematicians and conducted research with Dr. Howe, I learned there are exciting, rich new areas of mathematics which are relatively unstudied and ample career opportunities are available for pure math PhD graduates both inside and

outside academia. Furthermore, I realized that to succeed in a PhD program, it is essential to study a subject which is deeply motivating and inspiring. While pursuing research in mathematics and computer science, I have found myself to be deeply motivated to solve problems in pure mathematics. I have set my sights on a career in academia, as a researcher and instructor in the field of pure mathematics. While I decided to pursue math, I intend to leverage my strong computer science background and skills to aid in mathematical problem solving.

About the Author

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103.   **Investigating the Role of C5orf42 in Diabetic Kidney Disease**

Karah Hall

Faculty Mentor: Marcus Pezzolesi (School of Biological Sciences, University of Utah)

ABSTRACT

Diabetic kidney disease is a complication of diabetes and is the world's leading cause of end- stage kidney disease. Genetic factors are known to contribute to diabetic kidney disease susceptibility, however, despite intense effort, the identification of variants that underlie its risk has been challenging. Through the analysis of four pathogenic variants, we were able to investigate the potential role of the ciliary gene *C5orf42* in the susceptibility of kidney disease in patients with diabetes. *C5orf42* variants (I165Yfs*17 and S123F) were found to localize to different alleles, meaning that the biallelic carrier was a

compound heterozygous carrier. *C5orf42* variant V938Efs*27 was found to co-segregate with disease in a nuclear family. Lastly, *C5orf42* variant c.8300-G>C was found to be associated with three different isoforms.

INTRODUCTION

Diabetic kidney disease (DKD), also diabetic nephropathy, is a complication of diabetes that damages the blood vessels or glomeruli of the kidney and, therefore, affects the kidney's ability to remove waste products from the body. DKD is also the world's leading cause of end-stage kidney disease, which is life-threatening and has limited treatment options that include dialysis and/ or kidney transplantation. Identification of the genetic factors that contribute to DKD's risk may help identify those at risk of this disease and potentially lead to improved treatment or prevention strategies; these processes have been challenging thus far.[11]

The first published genome-wide association study on DKD susceptibility, published in 2009, was the Genetics of Kidneys in Diabetes (GoKinD) study.[3] Since then, there have only been a few reported genetic variants that have achieved genome-wide significance between single-nucleotide polymorphisms (SNPS) and this phenotype. Even fewer genetic variants have been replicated across different studies.[1-6] Disappointingly, none of these findings have been able to help improve diagnosis or remediation tactics for patients at risk of DKD. However, there is evidence of its strong genetic predisposition,

including an estimated heritability as high as 59%.5 Therefore, additional research is essential to better understand the genetic underpinnings of DKD and new approaches are necessary to increase the amount of gene discovery and make progress in this area of research.

With this goal in mind, Dr. Marcus Pezzolesi and his lab have used the Utah Diabetes Database, which contains electronic medical record data for more than 350,000 diabetic patients, the Utah Population Database, a unique population-based genealogy resource containing family histories and demographic data for 14 million individuals, and the Utah Kidney Study Biorepository, a biorepository of more than 2,000 diabetic and non-diabetic patients with kidney disease, to recently identify significant enrichment of 4 rare variants in *C5orf42* that are predicted to be pathogenic, including 3 putative loss of function (pLOFs) variants, in patients with diabetes and end-stage kidney disease. They were able to do so through targeted next-generation sequencing using a custom panel comprising 345 kidney disease-related genes in 222 participants of the Utah Kidney Study, including 98 individuals with non-diabetic kidney disease and 124 individuals with DKD.7

Interestingly, 25% of DKD patients positive for pathogenic or likely pathogenic variants were found to carry rare variants with minor allele frequencies of less than 0.01 in known ciliopathy-

associated genes, including 3 patients with type 2 diabetes and end-stage kidney disease found to harbor rare pLOF variants in *C5orf42* (c.8300-1G>C, I165Yfs*17, and V938Efs*27). Among these patients, two *C5orf42* variants (I165Yfs*17, a pLOF variant, and S123F, a variant predicted to be damaging by five of six computational prediction methods) were observed in one individual, suggesting that this patient could be a compound heterozygous carrier of rare damaging variants in this gene. Another carrier of a *C5orf42* pLOF variant has a family history of diabetes and DKD and has had their family members recruited to the Utah Kidney Study to investigate whether these variants co-segregate with disease in this family.

C5orf42, also known as *CPLANE1* and *JBTS17*, is associated with rare autosomal recessive ciliopathies characterized by cystic kidney disease.8-10 Importantly, in the presence of hyperglycemia, a state common in patients with diabetes, defects in cilia structure or function cause alterations in the kidney, including podocyte effacement, interstitial inflammation, and proteinuria.12 Based on these data, it can be hypothesized that defects in CPLANE1, the protein encoded by *C5orf42*, could contribute to DKD.

Although defects in ciliary genes have largely been associated with rare disease pathology, a recent study by Drivas and colleagues demonstrated the utility of a Mendelian pathway-

based approach to genomic association studies linking variants in ciliopathy genes to common complex disease, including several kidney-related traits.13 This paradigm

shift represents a major milestone in our understanding of the role of the primary cilium, a sensory organelle found in nearly every human cell, in human disease and, importantly, supports the role of ciliary genes in kidney disease pathogenesis.

These novel discoveries suggest that *C5orf42*, which is associated with rare autosomal recessive ciliopathies characterized by cystic kidney disease8-10, may also contribute to DKD. Here, we expand on this finding by further investigating the potential role of four pathogenic variants in *C5orf42* on the susceptibility of kidney disease in patients with diabetes.

METHODS

Sanger Sequencing-based Validation of C5orf42 Variants:

Four putative pathogenic variants in *C5orf42* that were identified in 3 DKD patients using targeted next-generation sequencing were analyzed as part of this study (Table 1).

First, genomic DNA was isolated from whole blood from all *C5orf42* variant carriers as well as recruited family members of UKS17D00134 using a standard phenol:chloroform DNA extraction protocol. Next, to confirm the presence of each variant in the carriers identified using next-generation sequencing, we preformed Sanger sequencing-based validation. PCR reactions were optimized to amplify each *C5orf42* variant using the primers listed in Table 2. PCR amplicons were purified using ExoSAP-IT Express (Applied Biosystems, Waltham, MA) and sequenced using an Applied Biosystems 3730xl by the University

of Utah's DNA Sequencing Core. The resulting chromatograms were then analyzed using the Sequencer 5.4.6 software by Genes Codon Corporation.

Sub-Cloning of I165Yfs*17 and S123F Variants:

PCR amplicons from the carrier of biallelic *C5orf42* variants (I165Yfs*17 and S123F) were also sub-cloned into *E. coli* using the TOPO TA cloning protocol with chemically competent TOP10 cells (Invitrogen, Waltham, MA) and plated on Luria Broth (LB) agar plates with ampicillin. Colonies were selected and inoculated in LB ampicillin media. Following inoculation, DNA was isolated using the QIAprep Miniprep Kit (Qiagen, Hilden, Germany) and Sanger sequenced to assess whether these variants localized to a single allele or to both alleles and whether this patient was a compound heterozygous carrier of these variants.

Segregation Analysis of the V938Efs*27 Variant:

In order to analyze the nuclear family of patient UKS17D00134 carrying the V938Efs*27 variant of *C5orf42*, isolated DNA was PCR amplified, purified, and submitted for Sanger sequencing. The resulting chromatograms were analyzed and segregation of this variant with diabetes and kidney disease were assessed in this family.

RNA Sequencing (RNASeq) Analysis:

To provide information about the transcriptome of each patient, total RNA was isolated from whole blood of carriers of each of the *C5orf42* variants, including the family of the carrier of the V938Efs*27 variant using the PAXgene Blood RNA Kit (Qiagen, Hilden, Germany). A total of 7 samples were submitted to the University of Utah's High-Throughput Sequencing Core for library preparation using NEBNext Ultra II Directional RNA Library Prep with rRNA Depletion Kit (New England Biolabs, Ipswich, MA) and RNA sequencing. The resulting data were analyzed using the Integrative Genomics Viewer (IGV) software.

Characterization of the c.8300-G>C Variant:

In order to authenticate observations made with IGV, which suggest multiple isoforms, PCR amplicons of the carrier of the splice variant c.8300-G>C were sub-cloned into *E. coli*, purified, and Sanger sequenced as described above. This variant was also further analyzed using the 3' RACE System for Rapid Amplification of cDNA Ends (Invitrogen, Waltham, MA), which utilizes the natural poly(A) tail found in mRNA as a nonspecific priming site for PCR to characterize mRNA transcripts. After amplifying cDNA from patient UKS17D00008 using the 3' RACE kit, the products were analyzed on an agarose gel and were also sub-cloned into *E. coli*.
These colonies were then Sanger sequenced.

The c.8300-G>C variant was further analyzed using the Genomis HSF Pro system, an online splice site prediction software. The genomic position of the variant was entered into

the Mutation Analysis tool, which can detect a variant's impact on splicing signals and acceptor and donor sites.

RESULTS

Confirmation of C5orf42 Variants in DKD Patients:

Our initial Sanger sequencing experiments confirmed our targeted next-generation sequencing results and that each DKD patient carried the rare *C5orf42* variant observed in these data (Figure 1).

Evidence for Compound Heterozygosity:

After the presence of the variants in each DKD patient was confirmed, the sequencing results of UKS17D00022, the carrier of biallelic *C5orf42* variants (I165Yfs*17 and S123F), from the TOPO TA cloning procedure determined that some *E. coli* colonies displayed variant I165Yfs*17, and others displayed variant S123F (Figure 2). No colonies displayed both variants or no variation at all. Therefore, these data show that these variants localize to different alleles or different chromosomes, meaning that this individual is a compound heterozygous carrier of rare variants in *C5orf42.*

*Co-segregation of Variant V98Efs*27 and DKD in Pedigree of UKS17D00134:*

Next, Sanger sequencing results from the nuclear family of patient UKS17D00134, carrying the V938Efs*27 variant of *C5orf42*, revealed that in addition to the proband being a carrier of this variant, the proband's father and sibling, both of whom have diabetes and kidney disease, were also confirmed to be carriers of this variant (Figure 3A). The proband's mother, who does not have diabetes or kidney disease, was not a carrier of the variant. These data demonstrate that the V938Efs*27 variant co-segregates with disease in this family (Figure 3B).

RNASeq Analysis Suggests Alternate Splicing due to c.8300-G>C Variant:

Upon analysis of the RNASeq data from the carrier of *C5orf42* pLOF splice variant (c.8300-G>C) with the interactive tool Integrative Genomics Viewer (IGV), two different isomers were discovered, one bearing a 30-bp deletion, and one a 27-bp deletion (Figure 4), suggesting that this variant results in aberrant splicing of *C5orf42* mRNA that may lead to potential alternative forms of the CPLANE1 protein.

Confirmation of Isoforms:

When attempting to authenticate the observations made above, Sanger sequencing results of DNA isolated from bacterial colonies carrying DNA from patient UKS17D00008 (the carrier of the c.8300-G>C splice variant) showed that some colonies displayed the wildtype sequence, some displayed a sequence with a 30 base pair deletion, and some displayed a sequence with a 27 base pair deletion (Figure 5A). This confirmed observations made with IGV and validated the existence of multiple isoforms caused by the variant. Upon further analysis of the splice variant via the 3'RACE kit, the agarose gel revealed multiple bands when the products were amplified with a gene specific primer in exon 40 of *C5orf42*, with two gene specific primers in exon 42 of *C5orf42*, and with nested gene specific primers in exon 40 and 42 (Figure 5B). This suggested the

existence of the multiple isoforms and was confirmed by Sanger sequencing the resulting gel band products, which again revealed the wildtype sequence, a sequence with a 30 base pair deletion, and a sequence with a 27 base pair deletion.

In Silico Confirmation of Alternate Acceptor Sites and C5orf42 Isoforms Due to c.8300-G>C Variant:

To further verify the multiple isoforms, the Genomis HSF Pro system results revealed that variant c.8300-G>C could create two alternate acceptor sites (Figure 6). Each of these sites included in their motifs the isoforms that were discovered through the subcloning and 3'RACE analyses, thereby confirming these results.

DISCUSSION

Through this research, a greater understanding of each variant of *C5orf42* identified in participants of the Utah Kidney Study was achieved. Patient UKS17D00022 was determined to be a compound heterozygous carrier of the I165Yfs*17 variant on one allele and the S123F variant on the other. This is important knowledge because this patient could transmit either allele to their children, therefore, causing them to inherit either variant. Its unclear, however, from this study whether one copy or both variants is needed to cause disease. Further research focusing on the rest of this patient's family and their family's history with diabetes and DKD may be useful in further analyzing the effects of

these variants in *C5orf42* on the susceptibility of DKD.

Additionally, the V938Efs*27 variant was found to co-segregate with diabetes and DKD in the family of patient UKS17D00134. This is also important knowledge because it shows a direct relationship between the gene *C5orf42* and the susceptibility of DKD. Continuing to analyze descendants of this family could be useful in further supporting this observation and could potentially warrant further genetic testing and genetic counseling of carriers of this variant in this family.

Lastly, two aberrant *C5orf42* isoforms were observed as a result of splicing variant c.8300-G>C; these were verified through Sanger sequencing of subcolonies, using 3'RACE amplification, and through *in silico* prediction using the splice site prediction tool. This discovery is important because it shows that this variant causes alternate splicing of *C5orf42* mRNA that results in multiple protein isoforms and may impact protein function. Further research on the functional impact resulting from the 30 base pair deletion and the 27 base pair deletion, which are predicted to result in altered protein isoforms lacking 10 and 9 amino acids, respectively, may be useful in determining how each isoform contributes to the susceptibility of DKD.

Another interesting finding that further supports the pathogenicity of two of the *C5orf42* variants is I165Yfs*17

localizes to the WD40 domain of the CPLANE1 protein and V938Efs*27 localizes near the WD40 domain in the non-cytoplasmic region of the CPLANE1 protein. Many potentially pathogenic variants of proteins in this domain have already been identified and linked to various human pathologies including neurological disorders, cancer, ciliopathies, and endocrine disorders.[14] Variants I165Yfs*17 and V938Efs*27 may result in similar effects. With this in mind, the genomic wildtype sequences (chr5:37,106,235-37,107,261 and chr5:37,106,235-37,108,169) and variant sequences of I165Yfs*17 and V938Efs*27 were translated into amino acid sequences using the ExPASy translating tool (https://web.expasy.org/ translate/), and protein modeling of the obtained amino acid wildtype sequences (1M-342T and 1M-967P) and variant sequences of I165Yfs*17 and V938Efs*27 (1M-180L and 1M-963M) was preformed using the Robetta protein structure prediction service (https://robetta.bakerlab.org) to predict the structure of the altered WD40 domain containing proteins relative to the wildtype sequence (Figures 7A and 7B). These differing structures may impact the protein's function and contribute to each of the patients' susceptibility to diabetes and DKD.

Our analysis of each of the identified *C5orf42* variants further characterized their potential role in DKD and further support the potential benefits of genetic research and how it is important to identify and study possibly deleterious gene variants like those found in *C5orf42* in order to diagnose diseases

more readily, identify individuals at risk of disease, and possibly identify cures for them in the future.

Additional functional analyses, including *in vitro* inactivation (knock-out) of *C5orf42* and the introduction (knock-in) of each *C5orf42* variant, are necessary to further examine the specific phenotypic effects of each variant of *C5orf42* and to further investigate the role of this gene in DKD. Transfection with short interfering RNA (siRNA), which binds to target mRNA and mediates its degradation, may be a means for *in vitro* inactivation and additional investigation of *C5orf42*'s function. Another gene silencing technique using the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 system may provide a better understanding of the gene's function, while also presenting an opportunity for the introduction of each variant of *C5orf42*. This system involves a guide RNA that matches the target gene along with a Cas 9 (CRISPR- associated) protein that acts as an endonuclease to cause a double-stranded DNA break.15 Once phenotypic effects of each variant are identified in cellular cultures via one of these techniques, proper treatment protocols can be developed in order to help patients who are genetically susceptible to DKD.

REFERENCES

1. Iyengar SK, Sedor JR, Freedman BI, et al. Genome-Wide Association and Trans-ethnic Meta- Analysis for Advanced

Diabetic Kidney Disease: Family Investigation of Nephropathy and Diabetes (FIND). PLoS Genet 2015;11:e1005352.

2. McDonough CW, Palmer ND, Hicks PJ, et al. A genome-wide association study for diabetic nephropathy genes in African Americans. Kidney Int 2011;79:563-72.

3. Pezzolesi MG, Poznik GD, Mychaleckyj JC, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. Diabetes 2009;58:1403-10.

4. Sandholm N, Forsblom C, Makinen VP, et al. Genome-wide association study of urinary albumin excretion rate in patients with type 1 diabetes. Diabetologia 2014;57:1143-53.

5. Sandholm N, Salem RM, McKnight AJ, et al. New susceptibility loci associated with kidney disease in type 1 diabetes. PLoS Genet 2012;8:e1002921.

6. Sandholm N, Van Zuydam N, Ahlqvist E, et al. The Genetic Landscape of Renal Complications in Type 1 Diabetes. J Am Soc Nephrol 2017;28:557-74.

7. Lazaro-Guevara J, Fierro-Morales J, Wright AH, et al. Targeted Next-Generation Sequencing Identifies Pathogenic Variants in Diabetic Kidney Disease. Am J Nephrol 2021;52:239-49.

8. Romani M, Mancini F, Micalizzi A, et al. Oral-facial-digital syndrome type VI: is C5orf42

really the major gene? Hum Genet 2015;134:123-6.

9. Wentzensen IM, Johnston JJ, Keppler-Noreuil K, et al. Exome sequencing identifies novel mutations in C5orf42 in patients with Joubert syndrome with

oral-facial-digital anomalies. Hum Genome Var 2015;2:15045.

10. Fleming LR, Doherty DA, Parisi MA, et al. Prospective Evaluation of Kidney Disease in Joubert Syndrome. Clin J Am Soc Nephrol 2017;12:1962-73.

11. Ma RC, Cooper ME. Genetics of Diabetic Kidney Disease-From the Worst of Nightmares to the Light of Dawn? J Am Soc Nephrol 2017;28:389-93.

12. Sas KM, Yin H, Fitzgibbon WR, et al. Hyperglycemia in the absence of cilia accelerates cystogenesis and induces renal damage. Am J Physiol Renal Physiol 2015;309:F79-87.

13. Drivas TG, Lucas A, Zhang X, Ritchie MD. Mendelian pathway analysis of laboratory traits reveals distinct roles for ciliary subcompartments in common disease pathogenesis. Am J Hum Genet 2021;108:482-501.

14. Yeonjoo K, Soo-Hyun K. WD40-Repeat Proteins in Ciliopathies and Congenital Disorders of Endocrine System. EnM Endocrinol Metab 2020; 302:1-13.

15. Redman M, King A, Watson C, King D. What is CRISPR/Cas9. et al. Arch Dis Child EducPract Ed 2016; 101:213–215.

Figure 1: Sanger Sequencing Data for Variants c.8300-G>C (top left), I165Yfs*17 (bottom left), S123F (top right), and V98Efs*27 (bottom right)

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Figure 2: Sanger Sequencing Data for Colony A (wildtype shown top left at base pair 261 and variant I165Yfs*17 shown top right at base pair 386) and Colony B (variant S123F shown bottom left at base pair 261 and wildtype shown bottom right at base pair 386)

Figure 3A: Sanger Sequencing Data for Proband's Father FS21003 (left), Proband's Sibling FS21001 (middle), and Proband's Mother FS21002 (right)

Figure 3B: Pedigree of Patient UKS17D00134 with Variant V938Efs*27

Figure 4: RNA Sequencing Data of UKS17D00008 in Integrative Genomics Viewer (IGV) Showing Isoforms with 27 and 30 Basepair Deletions (arrows) as a Result of the c.8300-G>C Variant (boxed)

Figure 5A: Sanger Sequencing Data for Colony A (wildtype shown top), Colony B (30 basepair deletion shown bottom left), and Colony C (27 base pair deletion shown bottom right)

Figure 5B: Agarose Gel Analysis of 3'RACE Products Showing Different Isoforms when Amplifying UKS17D00008 cDNA

Figure 6: Splice Site Prediction Tool for Variant c.8300-G>C, Showing Acceptor Site 1 (position: 37142465) with Motif ATATGGATGAAGGA (value: 71.45) and Acceptor Site 2 (position: 37142462) with Motif TGGATGAAGAAGAT (value: 66.86)

Figure 7A: Protein Modeling of Wildtype Sequence 1M-342T (left) and Variant

I165Yfs*17 Sequence 1M-180L (right)

Figure 7B: Protein Modeling of Wildtype Sequence 1M-967P (left) and Variant

V938Efs*27 Sequence 1M-963M (right)

Patient	Chr-Start	Variant	Ref	Alt	Exonic Function	Gnomad Frequencies
UKS17D00008	5-37142583	c.8300-G>C	C	G	splicing	3.9E-04
UKS17D00022*	5-37244554	I165Yfs*17	T	-	frameshift deletion	1.3E-04
UKS17D00022*	5-37244679	S123F	G	A	nonsynonymous	- - -
UKS17D00134	5-37213768	V938Efs*27	-	CT	frameshift insertion	6.5E-06

*this patient is a biallelic carrier of 2 pathogenic *C5orf42* variants

Table 1: Summary of *C5orf42* pLof Variants

Variant (s)	Primers	Length (base pairs)	Tm
c.8300-G>C	Foward: CTGGAACTGGAAAGGCTGAC	20	62
	Reverse: TGGGTTGCCAGTTGGAAATA	20	62
I165Yfs*17 and S123F	Forward: GTGGAAGGCAAGAGAACTGC	20	63
	Reverse: GCCGTGCCTGGATTGTATTT	20	63
V938Efs*27	Forward: CAAAGCTGCTGATTGGTGAA	20	61
	Reverse: GCCATGGGTGCTTATCTCAG	20	62

Table 2: Primers Used in the PCR Reactions of *C5orf42* pLof Variants

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104. **Lineage Tracing in Zebrafish with CRISPR Prime Editing**

Sahar Kanishka and James A. Gagnon (School of Biological Sciences)

Faculty Mentor: James Gagnon (School of Biological Sciences, University of Utah)

All embryos develop from a single cell. We use lineage tracing to map the relationships between individual cells and back to the initial founding cell. These lineage trees can help us understand how cells acquire their fates during normal development, and how that can go wrong in human disease. An emerging method for lineage tracing in embryos uses cellular barcodes. Cellular barcodes individually tag cells with a unique

set of mutations specific to that cell. As cell divisions occur, the barcode is passed on to the progeny cells and a lineage tree can constructed based on cells that share similar barcodes. The CRISPR-Cas9 system for gene editing is an ideal tool for creating a huge diversity of cellular barcodes in embryos. However, there are limitations with CRISPR-Cas9, including unpredictable indel formation and difficulties in recovering barcodes from cells. In this project, a modified CRISPR system known as prime editing will be applied in zebrafish, and utilized for lineage tracing. Prime editing allows for precise genome editing by inserting user-specified genetic sequences at a target site in the genome. I hypothesize that we can use prime editing to insert a huge library of user-specified barcodes into the genome of developing zebrafish. Because these barcodes are defined by the experimenter, they can be recovered at the end of the experiment using RNA in situ hybridization. In principle, lineage tracing with prime editing will allow us to discover the spatial arrangement of related cells in intact embryos and tissues. We hope to use lineage tracing with prime editing to understand the mechanisms of heart regeneration in zebrafish.

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105. Kinetics of H2 and Co2 Addition to a Ru Hydorgenation Catalyst That Converts Co2 to Formate

Gary Perea and Caroline T. Saouma (Chemistry)

Faculty Mentor: Caroline T. Saouma (Chemistry, University of Utah)

The hydrogenation of CO_2 to formate is generally thought to proceed via CO_2 insertion into a metal hydride. Indeed, (PNP)Ru(H)$_2$(CO) readily inserts CO_2 to give the corresponding (PNP)Ru(H)(CO)(OCHO) species (PNP = 2,6-bis(di-tert

butylphosphinomethyl)pyridine). However, we have observed that the formate species can also be formed from addition of H_2 to the ligand-bound CO_2 species, $(CO2\text{-}PNP)Ru(H)(CO)$. Given that ligand-bound CO_2 species are generally regarded as detrimental to catalysis, we sought to determine the mechanism of formate production. Ongoing kinetic studies on H_2 and CO_2 addition to the pertinent Ru metal species are being utilized to deduce the exact catalytic mechanism that are relevant for H_2 addition to carbonyl substrates.

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106.   **Research Reflection by Gary Perea**

Gary Perea

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I feel my experience as an undergraduate researcher has had an extremely important impact on not only my time in undergrad, but also on my future plans to attend graduate school. My research has given me the opportunity to put a lot of the knowledge I've acquired from my courses to practical use and helped me to connect that material to the real world. It's also allowed me to attend national conferences and present my research to my peers in the field which was an invaluable experience for my education. My time as an undergraduate researcher is something I will look back on fondly for many years and would highly recommend to anyone pursuing a degree in science.

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107. **CRISPR Perturbation of the Gene Regulatory Network That Specifies the Zebrafish Mesoderm**

Tejashree Prakash

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ABSTRACT

During early development, zebrafish cells undergo gastrulation, a process that establishes a cell's fate to develop mesoderm, a germ layer. Signaling pathways and key transcription factors (TFs) initialize a gene regulatory network

that specifies and diversifies mesoderm during gastrulation. Three TFs – *tbxta, tbx16,* and *noto* – are essential for the mesodermal gene regulatory network, affecting initiation and maintenance of mesoderm derivatives such as the notochord and tailbud. *tbxta*, *tbx16,* and *noto* regulate one another through a series of additive and antagonistic interactions during different stages of gastrulation. My objectives are 1) to adapt a single-cell RNA sequencing (scRNA-seq) computational pipeline to characterize the molecular profiles of these three transcription factors, and 2) to understand the effects in cell types when these transcription factors are perturbed through CRISPR mutagenesis. Using a CRISPR mutagenesis to scRNA-seq pipeline, the mutant embryos are compared to the control embryos to analyze differences in gene expression and cell abundance. However, a challenge for using scRNA-seq data exists in defining shared cell types across samples. To address this problem, I have developed an agnostic computational framework to transfer previously established cell type labels onto a perturbed sample and analyze how gene expression and cell type abundances are affected. Cell type labels from reference atlases are the input for the annotation framework, which transfers these labels onto a perturbed dataset's enriched cluster markers to identify its most likely cell types. By employing this framework, I identified the cell type changes across each perturbed condition in the developing embryos. My analysis recapitulates many known changes in cell abundance, such as loss of notochord and muscle, as well as novel changes in endoderm and aspects of ectoderm.

INTRODUCTION

Gastrulation is an essential process that defines the vertebrate body plan, possibly the most important event to occur in an organism's lifetime.

Gastrulation involves major cellular movements in a developing embryo, creating the first moment where stem cells commit to being progenitors for all cell types throughout the organism. Without this process, the cells of the embryo maintain pluripotency, where it is not confirmed to differentiate to a specific cell type. As the body of the embryo continues to develop, cells must obtain specific information as to what they will specify. This is the main purpose of gastrulation, to define the fate of cells in the embryo to commit to develop one of the three germ layers: ectoderm, endoderm, and mesoderm. In this overall process where a cell's fate is established to develop one of the three germ layers, there are two primary events that occur. First, a cell's fate is chosen to be ectoderm or mesendoderm; second, the mesendoderm cell's fate is divided into mesoderm or endoderm, which goes on to make its corresponding germ layer's derivatives.

The decision to induce mesendodermal cell types is defined by members of the Nodal signaling pathway (Campbell et al., 2005). Nodal signaling drives a pathway of receptor-transcription factor interactions that eventually regulates the expression of genes that are essential to developing the proper cell types in the appropriate regions of the embryo (Jones et al., 1995). Previous research has found that altering expression of Nodal signaling leads to large defects in mesendodermal

regions. For example, experimental increasing of Nodal expression causes cells that normally develop for ectodermal cell types to reroute to mesendoderm. Transcription factors that are downstream effectors of Nodal signaling, such as *cyc* and *sqt*, are actively expressed even before the beginning of gastrulation, suggesting they are foundationally involved in defining embryonic regions of mesendodermal development (Campbell et al., 2005). Essentially, transcription factors that are controlled through Nodal signaling specify the location of specific mesendodermal cell types to belong to the margin, prechordal plate, etc., based on its intensity of expression. Those cell precursors then receive signals from mesendodermal genes to go on to develop structures such as the notochord, which is specific to the posterior axis of the embryo.

The mesodermal germ layer specifies muscle, bone, somites, and the circulatory and urogenital systems. At this point, the cell has its fate determined to develop mesoderm, through interactions involving Nodal signaling, and now the cell will express a combination of transcription factors that will guide its further development into mesodermal derivatives. For example, cells in the posterior axis of the embryo will receive Nodal signals to determine its mesoderm status, then a transcription factor like *tbxta* will be expressed in this region to guide the development of the

notochord structure (Campbell et. al, 2005). These interactions between signaling pathways and transcription factors to finally establish a cell type is what is called a gene regulatory network. These interactions dictate the type and level of gene expression a cell experiences which in turn determines its function.

Three transcription factors, *tbxta, tbx16,* and *noto,* are known markers that guide the development and maintenance of mesodermal derivatives, acting as the mesodermal gene regulatory network. These three transcription factors have distinct but overlapping expression patterns in the mesodermal regions of the embryo. From previous research, *tbxta* and *noto* are known to be essential for development of the notochord, a cartilaginous spinal cord like structure along the dorsal side of the zebrafish. *noto* prevents the progenitor cells for notochord from differentiating as muscle cells (Campbell et al., 2005). In contrast, *tbx16* is expressed in mesodermal cells to differentiate muscles and somites along the dorsal axis of the fish as well. *tbx16* guides somite progenitors to the trunk of the fish, making it properly develop the corresponding somites of the trunk (Payumo et al, 2016). *tbxta, tbx16,* and *noto* have been studied for several years using different developmental biology and genetic techniques. Studies using techniques such as gene morpholinos have been used to study the function of these transcription factors by

perturbing the gene of interest and seeing its phenotypic affects. *tbxta* mutants present a shortened or lack of tail; *tbx16* mutants, also known as "spade tail", fail to differentiate somite and muscle cells appropriately in the tail, leading to a knot-like form in the tail; and *noto* mutants display a lack of notochord and fused somites (Schulte-Merker et al., 1994; Payumo et al., 2016; Stemple et al., 1996). The interactions between these three transcription factors remain unclear, whether they work synergistically, antagonistically, or perhaps a combination of the two. Thus, I study these three transcription factors using a set of novel tools to provide a different, molecular perspective of how these transcription factors interact to specify different layers of mesoderm.

Novel tools to study genetics and developmental biology have become more accessible to researchers, allowing classic developmental questions to be questioned in new perspectives. The CRISPR/Cas9 system is a gene editing technique that can introduce precise edits in an endogenous genomic locus of an organism (Ran et al., 2013). This genome editing method can be equipped in gene perturbation screenings, which is how it is applied in my experiment. Cas9 enzyme targets a site in the genome of the zebrafish complementing a guide RNA uniquely designed for the gene target of interest. The Cas9 then induces a double stranded break and excises this target sequence.

The removal of the target sequence ultimately induces a permanent mutation at that genomic site, causing a loss of function phenotype in the mutant embryo. CRISPR/Cas9 provides a new and relatively simpler method for genetic engineering that I have applied to study the mesodermal gene regulatory network through the induction of transcription factor perturbations.

Single-cell RNA sequencing (scRNA-seq) is a technology that enables the measurement of RNA transcripts from individual cells of an embryo, permitting analysis of the molecular compositions of each cell. In this method, mutant embryos of interest, acquired through CRISPR mutagenesis in my project, are dissociated into a state of single cell suspension, and submitted for 10X Genomics for scRNA-seq. The 10X Genomics method for scRNA-seq pools gel beads containing individual barcodes with each sample of single cells. A reaction takes place where the beads dissolve to tag mRNAs from each single cell with a unique barcode. These barcoded fragments are then assembled to generate a sequencing library for each mutant embryo. This constructed sequencing library maps each RNA read to its corresponding single cell, meaning that the library displays individual cell with its attributed molecular composition. The library is aligned to the zebrafish transcriptome of choice and put through a series of analysis pipelines in R to determine shared cell populations across

conditions, determining relative levels of cell abundance and gene expression. The combination of CRISPR/Cas9 technology with scRNA-seq provides avenues to understand the function of transcription factors through the perspective of cell abundance and gene expression, which is why I employ these technologies to study the mesodermal gene regulatory network.

Single-cell profiles require further manipulation to yield comprehensible information regarding the cell type composition of a given sample. As the output of single-cell sequencing experiments, single-cell datasets only maintain an identification of what sample condition each cell belongs to and what genes compose that cell, but not its specific cell type label. Previous genetic studies have compiled what genetic markers make up a particular cell type, but these datasets are not necessarily translatable to single-cell datasets in an automatic method. This creates a challenge in defining shared cell types across multiple perturbed samples efficiently, as manually doing so is time consuming and may lead to inaccurate labels. Therefore, I have created and applied an agnostic computational method that will transfer labels onto a perturbed single-cell dataset based on its composition of shared genetic markers. The ability to label cell types will identify what cell type the differences in cell abundance belong to, filling in that last space to have comprehensible molecular

composition information for single cells. Ultimately, the combination of the optimized CRISPR/scRNA-seq method with my computational framework will provide hypotheses for what cell type interactions are taking place when mesoderm cell types are perturbed in zebrafish embryos.

METHODS

Generation of gRNAs

Three gRNA sites were developed for each transcription factor, *tbxta, tbx16, noto,* and *tyr* by choosing target sites with high efficiencies and no overlap with one another on CHOPCHOP.

CRISPR/Cas9 Microinjection Mix

CRISPR injection mixes for *tbx16, noto,* and *tyr* were assembled with its corresponding gRNA in the following order: 1.5 µl 1M KCl (Sigma-Aldrich), 1.5 µl of the corresponding gRNAs, as generated above, 0.5 µl of phenol red (Sigma-Aldrich), and 1.5 µl of 20 µM CRISPR protein (Engen). The injection mix to target *tbxta* was assembled in an identical manner, except 1.0 µl of *tbxta* gRNA and 0.5 µl of pure H2O was used. The injection mixes were vortexed briefly to ensure proper mixing and kept on ice until used for embryo microinjection. During initial microinjection experiments, the *tbxta* injection mix was seen to have greater instances of cell death and toxicity. 3-4 puffs of injection mix were used, once the needle was calibrated, for *tbx16* and *noto,* and 2-3 puffs were used for *tbxta*.

Embryo Dissociation

Embryos were allowed to develop to 24 hours post injection, and those displaying a strong phenotype were chosen and placed in 1.5 ml Eppendorf tubes for dissociation and sequencing. The embryos were first dechorionated. Egg water was removed from each tube of embryos and replaced with 1 ml of 1mg/ml Pronase in 0.3X Danieau Buffer. The tubes were swirled gently for 3 minutes, and the Pronase solution was replaced with 1mL of 0.3X Danieau Buffer. This process of washing with Pronase then Danieau Buffer was repeated at least 4 times, until the chorions fell apart for all embryos. Once the embryos were dechorionated, the dissociation protocol was applied. The Danieau Buffer from the last dechorionation wash was replaced with 1mL of FACSmax solution. The tubes were triturated to homogeneity by pipetting up and down with p1000 pipette, until the tissue was properly broken up. Adding additional FACSmax solution, the tubes were incubated at 28℃ for 20 minutes, pipetting every 10 minutes to break up tissue. The solutions containing the embryos were passed through a 40um cell strainer to a new 50 ml Falcon tube. The cell pellets were centrifuged at 310 x g for 5 minutes, then the supernatant was discarded and replaced with 1 mL of PBS. The tubes were centrifuged additionally at 310 x g for 5 minutes, with the cell pellets resuspended in 1X PBS diluted with 0.5% BSA. The tubes of dissociated embryos

were kept on ice and submitted for 10X single-cell RNA sequencing.

PCR / Gel Electrophoresis

The genomic DNA was first extracted from the four mutant conditions through the "HotShot" DNA preparation method. First, egg water was pipetted out and replaced of 50 ul of Alkyl Lyse, incubated at 95℃ for one hour using a thermocycler, and 50 ul of Neutralizer was lastly added. Alkyl Lyse was developed with 25mM NaOH and 0.2mM EDTA, and the Neutralizer reagent was 40mM Tris-HCl. Aliquots of the primers for each TF were then made, consisting of 10uM of the stock primer previously developed for each TF's gRNA sequence. The PCR mix was created for each condition to a total volume of 25 ul: 1.25 ul reverse primer and 1.25 ul of forward primer, 3 ul of genomic DNA, 7 ul of pure H2O, and 12.5 ul of Q5 reagent. Along with the mutant genomic data, wildtype embryos were combined with each primer combination to allow for control analysis. The genomic data that has now been amplified were stained with 5ul of loading dye and loaded into a gel electrophoresis, along with the DNA ladder, in 120V for 25 minutes. A 1% agarose gel was created for the gel electrophoresis.

Single-Cell Data Import – Cell Ranger

The single-cell data retrieved from scRNA-seq submissions were aligned to the Lawson zebrafish transcriptome through Cell Ranger analysis

pipelines (Lawson et al, 2020). Each raw single-cell dataset's reads were appropriately mapped to the transcribed regions of the zebrafish genome through what was defined within the Lawson transcriptome. Cell Ranger ultimately outputs two files for each single-cell dataset: the "filtered_features_bc_matrix" with the mapped RNA reads and an html document summarizing the features of the dataset, such as cell count, number of reads, percent of reads mapped to genome, etc. The filtered_features_bc_matrix file for each sample, containing the barcodes, features, and matrix files, was the foundational data used in the Satija Lab's Seurat computational pipelines.

Single-Cell Data Analysis – Seurat Protocols

Cell Clustering

The "Guided Clustering Tutorial" from Seurat was applied to define clusters, or groups of cell populations, for the single-cell data of each perturbed condition (Hoffman, 2023a). Beginning with a Seurat object containing the "filtered_features_bc_matrix" file from Cell Ranger, the initial single-cell analysis requires a series of quality control (QC), filtering, and normalization methods. QC metrics, removing unwanted cells, were visualized through violin plots, where the cells are filtered to only include unique feature counts over 2,500. The data was then normalized, and a visualization determining the highly variable features was created using the *FindVariableFeatures* function. The highly variable features of a dataset are defined as features highly expressed in some cells while lowly expressed in others, important for further downstream analyses. Linear dimensional reduction (*RunPCA*

function) was performed, where these PCA scores determine the principle components in a variable feature set so like cells are clustered together. The function for creating clusters is *FindClusters*. The final step in this tutorial was applied to the single-cell datasets to visualize the clusters previously determined. UMAP, a non-linear dimensional reduction technique, colocalizes like cells, belonging to its cluster, together in a low-dimensional space.

Multiple Single-cell Dataset Integration

Seurat's "Introduction to scRNA-seq Integration Tutorial" was applied to integrate the perturbed conditions into one dataset (Hoffman, 2023b). The purpose of the integration was to create a new object that contained shared cell populations across multiple sample conditions. The integrated object creates ease in future analyses to identify differences in shared cell types across the different perturbed conditions. To achieve this, pairs of cells were identified across the multiple datasets that share the same biological state, or cell type, using the *FindIntegrationAnchors* function. These pairs of cells acted as the anchor points for congregating all the different sample's cluster data into a single dimensioned object, through the *IntegrateData* function. The now integrated object was put through a standard workflow for re-clustering and finally visualized as a UMAP plot. The *FindNeighbors* function specified what number of clusters the dataset should be forced to; the dimension yielding 45 clusters, unique to the integrated object, was used. Individual perturbed conditions were finally visualized in the integrated UMAP plot by using the *split.by* function, allowing differences in cell abundance between perturbed conditions to be visualized.

Label Transfer Computational Method

My code for these analyses are available at: https://github.com/Gagnon-lab/label-transfer-method

The integrated object determined cell populations shared across the different perturbed conditions but did not automatically allow those populations to be identified. The label transfer method was developed and applied to label these clusters using a previously established reference cell atlas that has defined the top markers expressed for a cell type. The first input for this computational method was the highly differentiated markers expressed for each cluster of the integrated object, obtained through the *FindAllMarkers* function in Seurat and filtered to only contain the genes that have a p value significance of less than 0.05. The second input was the Wagner Lab's reference cell atlas that defines the top 20 markers that defines a cell type for the 24hpf developmental stage (Wagner et al., 2018). Each input was formatted to be a compatible data frame, making a list of markers that defines each cell type of the cell atlas and each unlabeled cluster from the *FindAllMarkers* output. These two data frames were then compared to each other to create a match vector containing the magnitude of genes in common as well as a list of those genes. Ultimately, the highest match vector between a certain cell type and unlabeled cluster comparison will attribute that cell type to that cluster. The final outputs of the label transfer method were a heatmap displaying the magnitude of matches between the cell type reference and the cluster information as well as a table summarizing the highest match vectors; therefore, the label transfer method works to predict what the most likely cell type identification each previously unlabeled cluster is. The label transfer method has been adapted to a function in R called *label_transfer*. The function requires three inputs as .csv files: "annotation", "clusters", and "cell_type_names". "Annotation"

is the reference cell atlas of choice as a data frame, "clusters" is the *FindAllMarkers* output as a data frame, and "cell_type_names" is a data frame with only the name columns of the reference cell atlas. The *label_transfer* function outputs the heatmap and table summarizing cluster-cell type matches, as previously described.

Calculating Differences in Cell Abundance

Cluster-by-cluster, cell counts in each condition were logarithmically compared to the control condition, the *tyr*-injected condition. This was done by splitting up the integrated object condition-by-condition, and a log10 transformation calculation was done to create logarithmic comparisons. A data frame consisting of these comparisons was developed, positive values for increases in cell abundance compared to the control and negative values for decreases in cell abundance compared to the control. This data frame was employed to create a heatmap, using *melt* and *ggplot* R packages, to visualize the cell abundance changes, with the scale being a logarithmic scale of -1 to 1, with 0 being no change. Each cluster has an identity attributed to it through the previous label transfer method.

RESULTS
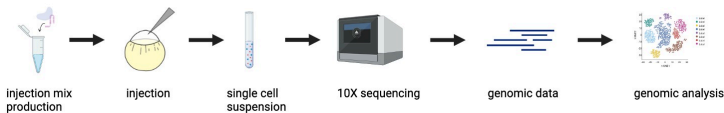
Figure 1: CRISPR Mutagenesis to Single-Cell RNA Sequencing Pipeline. An objective of this project was to optimize the pipeline of inducing CRISPR-mediated mutants in zebrafish embryos to obtaining single-cell RNA sequencing genomic data, and apply it to study the mesodermal gene regulatory network.

As previously stated, *tbxta, tbx16,* and *noto* are transcription factors (TFs) that initialize a gene regulatory network that specifies and diversifies mesoderm during gastrulation. The overarching objective of my project was to explore how these factors work together and individually to make different layers of mesoderm through the use of new developmental biology tools. I developed a CRISPR mutagenesis to scRNA-seq pipeline to study the function of these three TFs, as shown in Figure 1. My project can be summarized into a few steps: generation of mutants, dissociation to single cells and submission to 10X single-cell RNA sequencing, and the analysis of the scRNA-seq data. Perturbations were generated for *tbxta, tbx16,* and *noto* using a CRISPR-Cas9 system optimized to obtain mutants that recapitulate the TF's known mutant phenotypes. These embryos were dissociated into single cells and submitted for 10X single-cell RNA sequencing, yielding single-cell profiles of each mutant embryo condition for computational analysis.

1. Generation of Mutants

The first objective was to generate embryos for each condition, with *tyr*-injected embryos acting as a control experiment, as this gene only targets the pigmentation of the fish without influencing mesoderm or other cell types during development. These mutant embryos were generated for each condition using the CRISPR-Cas9 system. The CRISPR-Cas9 system uses an injection mix with pooled gRNAs that targeted each gene of interest with the CRISPR protein to essentially cause loss-of-function mutations for each condition (Figure 2a). The gRNAs I designed for each TF were chosen through CHOPCHOP.
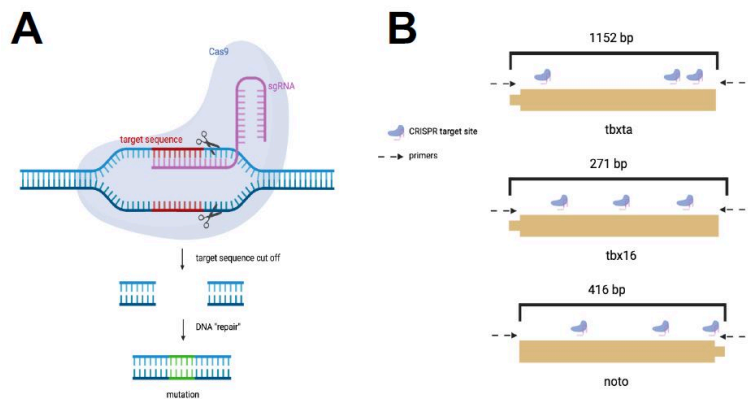
Figure 2: CRISPR Cas-9 System for Mesoderm Perturbations.
I used the CRISPR-Cas gene editing system to induce mutants in the three mesoderm transcription factors. **A.** The CRISPR system targets unique gRNA sequences to induce mutations at that genomic site. **B.** A gRNA sequence with three cut sites were designed for each transcription factor.

A sequence of varying base-pair length was selected for *tbxta, tbx16,* and *noto* with three target sites per sequence based on their assigned mutability efficiency scores (Figure 2b). In total, three gRNAs were pooled together for each of the four genes and used to create the gene's corresponding injection mixes. The injection mixes were individually created to perturb each gene of interest. Once the embryos were injected with the injection mix, mutant embryos were allowed to develop until 24 hours post fertilization (hpf) and were then observed for the expected phenotypes.

Figure 3a demonstrates the mutant phenotypes of the three gene perturbations, *tbxta, tbx16,* and *noto*. The *tbxta* mutants displays a shortened/lack of tail; *tbx16* mutants display a spade tail, where muscle cells are over-differentiated in the tail;

mutants of the *noto* condition display a lack of notochord and fused somites; and *tyr* mutants only have pigmentation loss as their observed phenotype. Through my experiments, I found that a consistent concentration used for the gRNAs across the conditions did not always yield healthy embryos with its expected mutant phenotype.
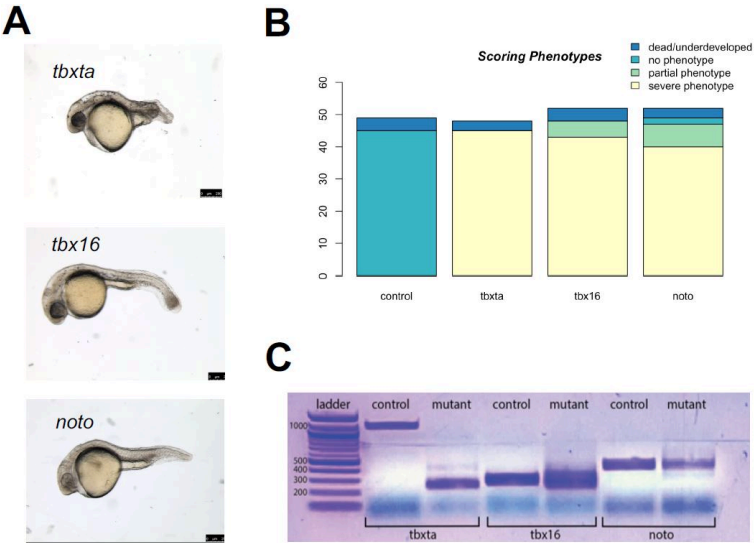
Figure 3: Generation of CRISPR mutants. A. *noto, tbxta,* and *tbx16* mutants with the expected mutant phenotype was generated through the CRISPR-Cas9 system. **B.** After calibration, my CRISPR injections yielded a high success rate of severe mutant phenotypes: *noto* injections showed a success rate of 90.38%, *tbx16* with 92.31%, and *tbxta* with 93.75%. **C.** The mutants were confirmed to have mutations in the genomic level through the difference in band sizes between control and mutant embryos per condition corresponding to the CRISPR cut sites previously designed.

Therefore, calibrations were performed in a series of injections with varying gRNA concentrations for each gene. The standard protocol for the injection mix called for 1.5 ul of gRNA, but

that led to embryo death and toxicity for *tbxta* mutant embryos, while *tbx16* and *noto* embryos did not display its appropriate mutant phenotype at 24hpf. After a series of calibration experiments, I determined that *tbxta* called for 1.0 ul gRNA with 0.5 ul purified H2O with only 2 puffs of injection mix during the injection, and *tbx16* and *noto* required 1.5 ul gRNA with 3-4 puffs during injection. My final calibration for the injection mixes of the CRISPR-Cas9 protocol yielded a high success rate, as displayed in Figure 2c; only few of the injected embryos were dead and underdeveloped or were observed as wildtype, and a significant majority demonstrated a severe, expected phenotype. The success rates were quite high across all conditions: *noto* with 90.38%, *tbx16* with 92.31%, and *tbxta* with 93.75%.

The CRISPR-Cas9 protocol I optimized yielded mutant embryos with the appropriate phenotypes, but I wanted to confirm that the mutations had occurred in the genes I targeted, using PCR and gel electrophoresis. Gel electrophoresis was used to visualize the mutations and differences present in the gene expression between wildtype embryos and mutant embryos. I performed a polymerase chain reaction to amplify the genomic DNA extracted from each condition's mutant embryos before being visualized on the gel. I generated primers to correspond to each gRNA's cut sites that were previously designed (Figure 1b). These primers were applied to wildtype and mutant embryos; the mutations that would have occurred in the mutants would have been in the genomic locations specified by the primers. In each perturbation condition, the gel visualized differences in DNA band sizes between the control and mutant, suggesting that mutations occurred in the genomic level (Figure 2e). These differences in band sizes corresponded to the gRNA cut sites:

there is a large difference between the bp length of the *tbxta* mutant band versus the control band, which corresponds with the large distance between the first two target cut sites in the gRNA designed for *tbxta.* Similarly, the relatively equal spacing between the three cut sites in the *noto* and *tbx16* gRNAs are consistent with the various and similar length of band sizes presented in the mutant columns.

1. Single-Cell Analysis

10X Genomics scRNA-sequencing outputs raw genomic data for each of the perturbed conditions that first had to be aligned to a zebrafish transcriptome which aligns the RNA reads to the appropriate genomic location. I chose to align the raw single-cell data to the Lawson transcriptome, which was stated to better quantify 3' and 5' UTRs to represent the zebrafish genome more thoroughly (Lawson et al, 2020). The now aligned RNA reads were put through Seurat's single-cell analysis workflow (Figure 4a).
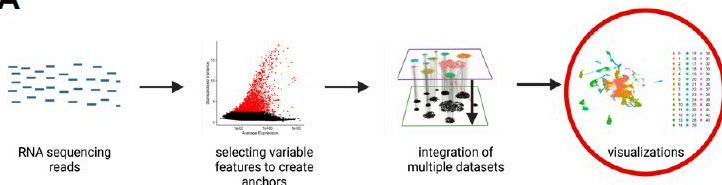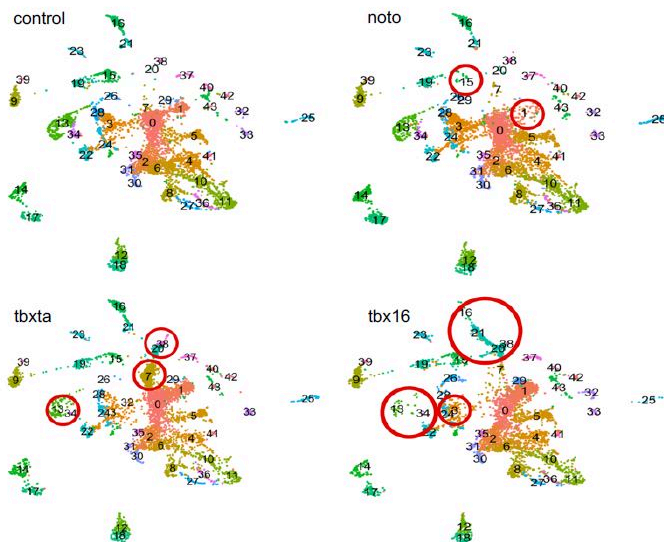
Figure 4: A single-cell CRISPR gene perturbation analysis workflow. The RNA sequencing reads are put through the Satija Lab's Seurat workflow for clustering and integration (described in methods). Ultimately visualizations can be generated to understand the cell populations and how it differs between perturbed conditions.

Through a series of quality control, filtering, and normalization methods, single cells in each sample were clustered together based on similar gene expression to create sub-populations or "clusters". A data integration across all four samples was also implemented to identify cell types present in all conditions, enabling the detection of similarities and

differences between samples; essentially, I developed clusters that aggregate cells with similar gene expression across all the sample conditions (control, *tbxta, tbx16,* and *noto*). UMAP visualizations were generated for the integrated object, split up by sample condition, as shown in Figure 4b. Visually, I was able to see differences in density of clusters in mutant conditions compared to the control, as demonstrated by the red circles in panel 4b.

However, the arbitrary numbered identification given by Seurat does not attribute any information regarding cell types to each cluster, so these visual differences and similarities in cell abundance did not initially yield useful information. Reference cell atlases exist that identify the top marker genes that establish a cell type, which could be manually applied to sample data, but it is inefficient and not precise. Therefore, I developed a label transfer method that transfers cell type labels of an existing reference cell atlas to my unlabeled integrated single-cell data (Figure 5). I developed this framework around the two inputs I was having difficulty putting together: the FindAllMarkers output that lists the highly differentiated markers for each cluster of the integrated object and the reference cell atlas that contains the top 20 markers that establish a cell type. I used the Wagner annotation for 24hpf zebrafish embryos (Wagner et al., 2018). This annotation was recently developed by the Wagner Lab to profile the top 20 genetic markers that establish cell types of zebrafish at the 24hpf time point; because of this time point, I decided to use this annotation as my reference cell atlas. I created a loop that essentially compares each list of markers for a cell type against each list of markers for an unlabeled cluster. The loop determines a match vector that is the number of matches between each cell type and each cluster, theorizing that a high

match vector equates a most likely cell type identification. To visualize these match vectors, a heatmap, as displayed in Figure 5, was generated to display the magnitude of matches between the two data frames. Finally, these clusters can then be renamed in the object and applied to the UMAP plot, so the cell abundance differences have a cell type attribution.
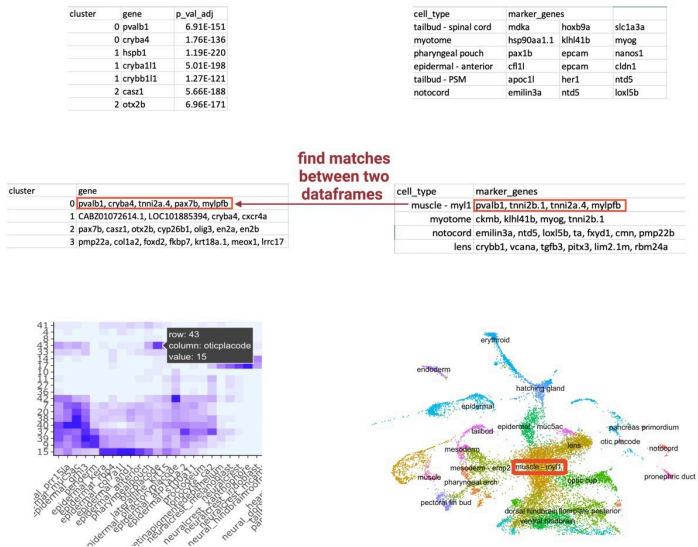
Figure 5: Label Transfer Schematic. An agnostic computational framework that transfers cell type labels from a reference atlas onto a perturbed sample's dataset. The final output is a heatmap visualizing the magnitude of correlation between the query sample's highly differentiated markers for each cluster and the reference atlas's markers for a defined cell type.

Stated previously, the label transfer method uses the highly differentiated markers for each cluster generated from the integrated dataset workflow. In this Seurat workflow, the dimensionality chosen when the *FindClusters* function is used forces Seurat to create a certain amount of clusters for a dataset. For example, using *resolution = 1.5*, which is Seurat's standard, yields a greater number of clusters while *resolution = 1.0* forces a lower number of clusters; this resolution refines what is considered enough for cells to have similar gene expression and be in a same sub-population. To test the robustness of my label transfer framework when the cluster input varies, I tested three different scenarios in which the number of clusters, and therefore what cells constitute each cluster, are different (Figure 6a).

A

B

Figure 6: Cluster/Marker Distribution. A. I tested different dimensionalities of clusters and its corresponding markers to see how identification of cell types vary. A mean amount of clusters doesn't force the cells to under/over cluster, meaning the cells that don't share as much gene expression do not congregate together. **B.** Using the 45 clusters, I generated each cluster's cell type identification, as shown in the heatmap output of the label transfer framework. The x-axis is the cell type labels from the reference atlas and the y-axis is the unlabeled clusters. A darker hue of square indicates a larger, or better, match magnitude, with the maximum being 20 and minimum of 0.

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The first scenario is where I used *resolution = 0.5* to force a lower number of clusters compared to the standard at 28 clusters. The second scenario used the standard of *resolution = 1.5* to obtain 45 clusters, and the third scenario used *resolution = 3.2* to yield 67 clusters. After obtaining the *FindAllMarkers* data set for each of these scenarios, I individually inputted them to my label transfer method with the Wagner reference to see how the identifications of cell types are altered. I quantified having a better identification by determining how many clusters demonstrated a match vector of at least 10, as a match vector of 20 would be considered a perfect match based on the construction of the Wagner reference. Generally, forcing the integrated object to have a cluster dimensionality closer to Seurat's standard allowed for better identification of clusters with cell types. From this determination, I chose to use the *resolution = 1.5*, with 45 clusters, for further single-cell analysis. I finally was able to determine the cell type labels of the subpopulations of my sample dataset, as shown in the heatmap of Figure 6b.

- Quantifying Cell Abundance Differences

With the cell type identities of my sample dataset determined, I wanted to quantify the visual differences I had previously seen in cell type abundances for each genotype. To do this, I used a log10 transformation calculation, comparing each mutant condition to the control condition to see if there were increases, decreases, or no changes in cell abundance in the mutants. Figure 7 displays this information as a heatmap.
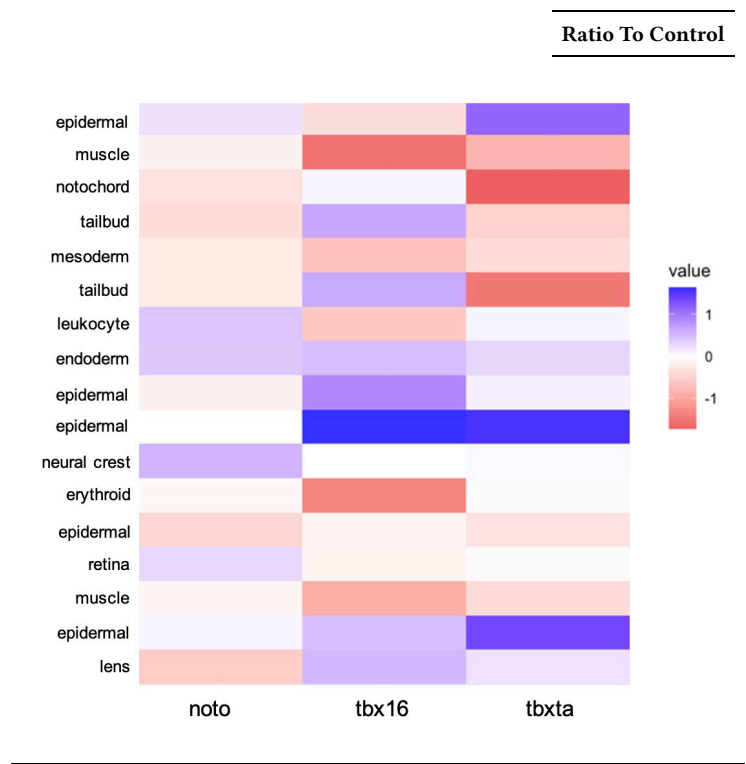
Figure 7: Quantifying Differences in Cell Abundance. A log10 difference was used to compare each mutant condition to the control. The scale is -1 to 1, with -1 being a decrease in mutant's cell type compared to control and +1 being an increase in mutant compared to control.

There are differences in cell abundance that were expected by the nature of the perturbation and what we know of these transcription factors previously: *tbx16* had a large decrease in muscle cells which corresponds to cells creating the knot-like formation in the tail, rather than differentiating as muscle, also

correlating with the increase in tailbud cells. *tbxta* presented a decrease in notochord and tailbud cells that matches with its loss of tail phenotype. *tbx16* has also been speculated to have interactions in the development of the heart, making the loss of erythroid cells an interesting observation (Griffin & Kimelman, 2002). Compared to the other two mutant conditions, *noto* overall displayed a less severe amount of cell abundance differences when compared against the control. Throughout the mutant conditions, all mesoderm cells decreased in comparison to the control, which is expected in these overall mesoderm-perturbed conditions. There is a shared increase in epidermal cells in all the mutant conditions, which reflects a potential phenomenon, perhaps cells are forced to develop other cell types such as epidermis when they cannot form mesendoderm. This observation could support the reasoning behind cells developing to ectoderm versus mesendoderm.

An interesting observation that has not been previously seen is the shared increase in endodermal cells. Compared to all other cell types that show an increase or decrease in cell abundance, endoderm has the greatest logarithmic increase in all three mutant conditions. *noto* was determined to have a 0.39-fold increase in cell abundance, *tbxta* with 0.30, and *tbx16* with 0.46. Because the increase in cell abundance seemed significant relative to differences seen in other cell types, I chose to further investigate this observation. I have preliminarily hypothesized that cells might default to endoderm when the decision to develop mesoderm is being denied. It was difficult to determine the actual significance of this observation, therefore I decided to use different experimental methods to test my hypotheses. Right now, I am performing an in-situ hybridization with the *sox17* RNA probe, a classic marker for endoderm. I believe this validation

will be further evidence to either support or not support my hypothesis.

DISCUSSION

The broad objective of my project was to understand how the mesodermal gene regulatory network, consisting of *tbxta*, *tbx16,* and *noto*, functions to specify different layers of the mesoderm during early development. As stated previously, these three transcription factors are classic markers for the mesodermal layer, having been studied to see how they each contribute to developing key mesodermal derivatives such as the notochord and somites. I wanted to apply the novel technologies of CRISPR gene editing and single-cell sequencing to answering this question, and essentially shine a new perspective on answering a classic question. I developed and optimized several protocols, experimental and computational, for the combining of CRISPR mutagenesis with scRNA-seq techniques in this pursuit to study the mesoderm. The model I have developed streamlines the generation of mesodermal mutants using CRISPR and the analysis of the molecular profiles of individual cells within each mutant. Developing this model first began with trying to recapitulate what is known about these transcription factors, establishing the efficacy of this combination of methods. The mutants that were generated using the CRISPR system was done with the goal to reflect each TFs already established mutant phenotype in previous literature, where older genetic tools were used such as morpholinos to create those mutants. Similarly, I was expecting the mutant phenotypes to be reflected in the single-cell data,

in which the cell types that were visually perturbed were also affected in the single-cell level.

Like CRISPR gene editing, single-cell sequencing analysis required a lot of troubleshooting through experiencing the limitations of the novelty of the technology. I experienced this greatly in trying to identify the sub-populations of my samples, so I could efficiently understand how different cell types are influenced after undergoing a perturbation. I initially tried to label the sub-populations of my samples by hand, comparing the top 20 expressed markers within each cluster to the top 20 markers that establish a cell type; this method was quite tedious and allowed for error. This difficulty in doing so led me to develop a computational method that transfers labels in an automatic and unsupervised fashion. The label transfer method was also written in a matter where it is compatible with any reference cell atlas and any *FindAllMarkers* output, so it is transferable to others who would like to quickly identify the sub-populations in their single-cell dataset regardless of what timepoint or type of embryos they are working with. I adapted the framework to be a function in R and is downloadable through the Gagnon Lab's GitHub, which is linked in the appendix.

The cell abundance differences I observed from my single-cell data demonstrated known changes in cell types, like the decrease in notochord for *tbxta* and decrease of erythroid cells in *tbx16*. I also observed changes in cell types that haven't been established in previous literature, such as the general increase in endodermal cells across all the mesoderm-perturbed conditions. However, it was difficult to determine the significance of these changes I observed, as my experiment did not allow for replicates of single-cell data. This meant that calculating significance using statistical methods was not a

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fruitful avenue to go down; instead, I chose a few hypotheses, one being the endodermal hypothesis, and am finding different ways to test them. The experimental pathway I have chosen to confirm these hypotheses is to visualize endodermal gene expression through in-situ hybridization (Thisse & Thisse, 2008). The in-situ hybridization method allows me to visualize the RNA of a gene of choice in the mutant embryos I have generated. I am using a *sox17*, a classic endodermal marker, probe to visualize its gene expression in the mutant and control embryos to see if there is an increase in endodermal expression.

I hope to use the optimized methods I have developed using CRISPR and scRNA-seq technologies to further understand the mesodermal gene regulatory network. The beginning of this project was founded in exploring the different interactions *tbxta*, *noto,* and *tbx16* have with each other, specifically how they worked synergistically and antagonistically to overall develop the mesodermal layer. I would also like to identify redundancies in this network by inducing double and triple mutants using this optimized pipeline. I believe introducing double and triple mutants will allow for me to observe the presence of synergistic and antagonistic interactions between the pairs of transcription factor perturbations. Beyond *tbxta*, *noto,* and *tbx16*, there are other pathways and genes that influence mesoderm specification, it would be interesting to apply the methods I have developed to different genes and transcription factors to understand early development of the zebrafish.

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Thank you so much to Dr. James Gagnon for guiding me through the project and introducing me to the world of

genetics and developmental biology research. Thank you for encouraging and supporting me academically and personally throughout my undergraduate career. I would also like to thank Sheng Wang and Clay Carey for providing me with your support in teaching me how to run lab protocols and troubleshoot my computational work. I could not have done it without all of you.

REFERENCES

1. Campbell, A., Anderson, W. W., & Jones, E. W. (2005). Molecular Genetics of Axis Formation Zebrafish. In *Annual Review of Genetics* (pp. 579–581). Essay, Annual Reviews.

2. Griffin, K. J., & Kimelman, D. (2002). One-Eyed Pinhead and Spadetail are essential for heart and somite formation. *Nature cell biology*, *4*(10), 821–825. https://doi.org/10.1038/ncb862

3. Hoffman, P., & Satija Lab. (2023, March 27). *Seurat – Guided Clustering Tutorial*. • Seurat. Retrieved from https://satijalab.org/seurat/articles/pbmc3k_tutorial.html

4. Jones, C. M., Kuehn, M. R., Hogan, B. L., Smith, J. C., & Wright, C. V. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development*, *121*(11), 3651–3662. https://doi.org/10.1242/dev.121.11.3651

5. Lawson, N. D., Li, R., Shin, M., Grosse, A., Yukselen, O., Stone, O. A., Kucukural, A., & Zhu, L. (2020). An improved zebrafish transcriptome annotation for sensitive and comprehensive detection of cell type-specific genes. *eLife*, *9*, e55792. https://doi.org/

10.7554/eLife.55792

6. Payumo, A. Y., McQuade, L. E., Walker, W. J.,
    Yamazoe, S., & Chen, J. K. (2016). Tbx16 regulates
    hox gene activation in mesodermal progenitor
    cells. *Nature chemical biology*, *12*(9), 694–701.
    https://doi.org/10.1038/nchembio.2124

7. Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott,
    D. A., & Zhang, F. (2013). Genome engineering using
    the CRISPR-Cas9 system. *Nature protocols*, *8*(11),
    2281–2308. https://doi.org/10.1038/nprot.2013.143

8. Schulte-Merker, S., van Eeden, F. J., Halpern, M. E.,
    Kimmel, C. B., & Nüsslein-Volhard, C. (1994). no tail
    (ntl) is the zebrafish homologue of the mouse T
    (Brachyury) gene. *Development (Cambridge,
    England)*, *120*(4), 1009–1015. https://doi.org/10.1242/
    dev.120.4.1009

9. Stemple, D. L., Solnica-Krezel, L., Zwartkruis, F.,
    Neuhauss, S. C., Schier, A. F., Malicki, J., Stainier, D.
    Y., Abdelilah, S., Rangini, Z., Mountcastle-Shah, E., &
    Driever, W. (1996). Mutations affecting development
    of the notochord in zebrafish. *Development
    (Cambridge, England)*, *123*, 117–128. https://doi.org/
    10.1242/dev.123.1.117

10. Thisse, C., & Thisse, B. (2008). High-resolution in situ
    hybridization to whole-mount zebrafish
    embryos. *Nature protocols*, *3*(1), 59–69.
    https://doi.org/10.1038/nprot.2007.514

11. Wagner, D. E., Weinreb, C., Collins, Z. M., Briggs, J.
    A., Megason, S. G., & Klein, A. M. (2018). Single-cell
    mapping of gene expression landscapes and lineage
    in the zebrafish embryo. *Science (New York,*

N.Y.), 360(6392), 981–987. https://doi.org/10.1126/science.aar4362

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108. **Research Reflection by Tejashree Prakash**

Tejashree Prakash

Faculty Mentor: James Gagnon (School of Biological Sciences, University of Utah)

My research experience really shaped my entire undergraduate career. I had the opportunity to craft my own project with just the supervision of Jamie, my PI, and carry it through for almost two years. Jamie was an integral part of my research experience, he believed in me to bring me into his lab when I was just a sophomore with no prior experience, and continued to encourage me as I grew my skills. It gave me so many opportunities to present my research and really develop my skills that go beyond biological science research such as presenting, creating, collaborating, etc. My research experience has opened a lot of doors for me now that I am a graduating Senior. I am branching into new fields of health science and biological science, and I am really excited to see how the tools

I have garnered throughout my undergraduate research career will be applied to explore new disciplines.

About the Author

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109. **The Suppression of Signal-Switching Defects in the Escherichia Coli Serine Chemoreceptor**

Ana Rowe and John Parkinson (School of Biological Sciences)

Faculty Mentor: John Parkinson (School of Biological Sciences, University of Utah)

ABSTRACT

The model bacterium *Escherichia coli* contains a chemotaxis system that allows the cell to change its swimming behavior in

response to its environment. As the cell swims, transmembrane chemoreceptors detect concentration changes in attractant and repellent compounds and transmit signals across the inner membrane that elicit an appropriate locomotor response. Previous work identified an amino acid near the cytoplasmic tip of the receptor (F396) that plays a critical role in enabling Tsr to modulate its signal output in response to a serine stimulus. The objective of my project is to characterize second-site mutations that rescue the chemotactic ability of F396 receptor mutants. These suppressors presumably enable mutant receptors to undergo the conformational changes necessary for serine sensing and signaling. To identify such suppressors, I subjected plasmids that encode Tsr-F396G (glycine) or Tsr-F396W (tryptophan) mutant receptors to random mutagenesis and selected for mutant plasmids that promoted improved serine chemotaxis. DNA sequence analysis of the revertant plasmids showed that most of the suppressor mutations lie near the hairpin tip of Tsr. I obtained few F396W revertants and none with substantially improved function in contrast to F396G, which gave rise to more revertants with better chemotactic ability. In subsequent experiments, I determined the expression levels of the doubly mutant Tsr proteins and characterized their signaling properties with *in vitro* FRET-based kinase assays. My findings served to identify Tsr structural features important for signal control of the cytoplasmic tip and enabled me to develop a more complete model of transmembrane signaling by the Tsr protein.

INTRODUCTION

In the microbial world, as in all of life, organisms detect and process information about their environment. To internalize meaningful messages of external stimuli, microorganisms require extraordinarily sensitive sensory systems. Such

systems enable motile microbes to move toward favorable chemical environments and away from noxious chemicals, a behavior known as chemotaxis. Specialized transmembrane receptors facilitate sensing and responding to these environmental cues. These receptors, called methyl-accepting chemotaxis proteins (MCPs), initiate a communication cascade of signaling steps that ultimately control the cell's locomotion.

Escherichia coli is a motile bacterium that swims by rotating its flagellar filaments. The rotary motors can turn in both clockwise (CW) and counterclockwise (CCW) directions (Figure 1A). CCW rotation enables the flagellar propellers to form a bundle that pushes the cell forward in a "run". A CW reversal of one or more motors causes the bundle to disperse and the cells to execute random turning motions, termed a "tumble". When swimming in the absence of a chemical gradient, the flagellar motors switch unpredictably between CW and CCW rotation, causing the cell to move about in a random-walk fashion. In an attractant gradient whenever the cell heads up-gradient, it senses that attractant concentration is increasing over time and this information produces a signal that lowers the probability of the next tumbling event. Thus, *E. coli* cells move up-gradient in a biased random walk fashion (Figure 1B). *E. coli* has five MCP-type chemoreceptors that sense different chemicals. The serine chemoreceptor, Tsr, and the other receptors function as membrane-spanning homodimers with a cytoplasmic signaling domain (Figure 2; Parkinson et al, 2015). Three receptor dimers assemble trimers of dimers through association of their cytoplasmic tips. The trimer tips provide an

interaction surface for an adaptor protein, CheW, that in turn couples an autokinase, CheA, to chemoreceptor control (Parkinson et al, 2015).
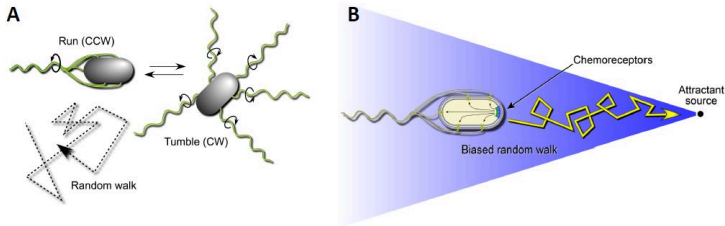
Figure 1: Swimming behaviors of *E.coli*. A. Transmembrane chemoreceptors detect changes in their surrounding environment and transmit signals that control the cell's flagellar motors. In the absence of a chemical gradient (right) the cell will swim in a tumbling motion to seek out nutrients. B: Transmembrane chemoreceptors detect changes in their surrounding environment and transmit signals that control the cell's flagellar motors. In the presence of an attractant source, bacteria such as *E. coli* can modulate their swimming behavior to favor a forward swimming motion.

Receptor signaling complexes have kinase-ON and kinase-OFF activity states (Figure 3).

When no attractant is bound to the receptors, CheA autophosphorylates, using ATP as the phosphodonor. In this kinase-ON output state, CheA transfers its phosphoryl groups to CheY molecules, which bind to the flagellar motors to promote CW rotation (Figure 3). Phospho-CheY has a short half-life due to a phosphatase partner CheZ, which enables the cell to rapidly change its swimming behavior upon detecting a change in chemoattractant level (Silversmith et al, 2003).

Attractant-bound receptors undergo a conformational change that propagates to the associated CheA and stops its autokinase activity (Parkinson et al, 2015). In this kinase-OFF state, phospho- CheY levels quickly plummet, through CheZ activity, and the flagellar motors adopt their default CCW direction of rotation.

Because of their small size, collisions with water molecules (Brownian motion) can knock *E. coli* cells off-course, so they can only traverse attractant gradients in a biased random-walk fashion. To effectively explore their environment for nutrients, the cells must make temporal comparisons of attractant concentration as they swim about. Reversible covalent modifications of the receptor molecules provide a short-term "memory" of the recent chemical past. Enzymes CheB and CheR catalyze those modifications to adjust receptor output to prevailing chemical conditions (Figure 4). CheR, a methyltransferase, interacts with kinase-OFF receptors and converts specific glutamyl residues to glutamyl methyl esters, which shifts signal output toward the kinase-ON state (Lai et al, 2017). Conversely, the CheB interacts with receptors in the kinase-ON state and shifts them toward kinase-OFF output by hydrolyzing the methylated glutamyl residues (Parkinson et al, 2015). As such, this system acts as a "memory" for the cell so that it can continue to move up the chemical gradient.
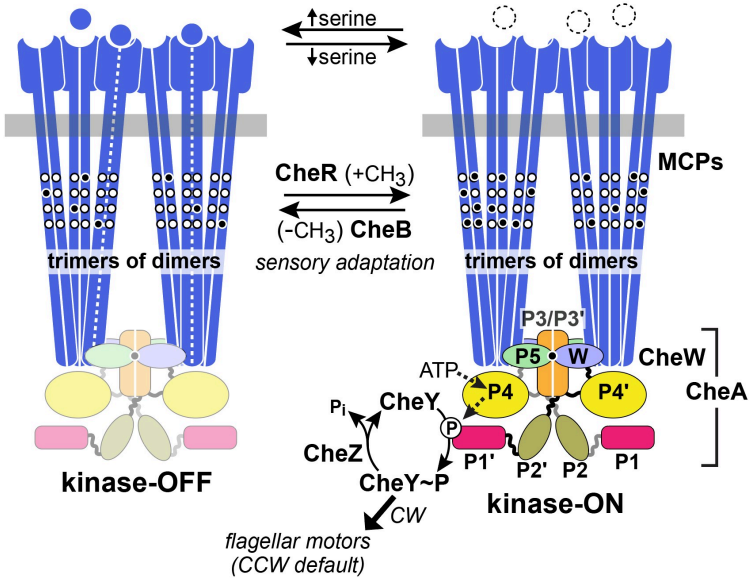
Figure 2: Two-state model of receptor signaling and the chemotaxis phosphorelay pathway. In the absence of a stimulus (right), CheA autophosphorylates and the reaction promotes signaling states that enhance CW flagellar rotation. When an attractant is bound, CheA is dephosphorylated to promote signaling states that augment CCW flagellar rotation. Binding of a ligand or removing methyl groups shifts the receptor from the kinase-on to the kinase-off state. Attractant release and addition of methyl groups shift the receptor from kinase-off to kinase-on (Parkinson et al, 2015).

MCPs cluster at the cell poles to form large cooperative receptor signaling arrays (Pinas et al, 2022). Array formation enhances chemoreceptor sensitivity by enabling receptors to share and cooperatively act on stimulus information. Signaling interactions between arrayed receptors produce highly cooperative responses to attractant stimuli such as serine (Briegel et al, 2014). Additionally, homodimers of MCPs such

as Tsr can assemble into mixed trimers of dimers with other chemoreceptors such as Tar (aspartate and maltose receptor), which further enhances the sensing capabilities of the cell to modulate kinase activity (Ames et al, 2002).

Tsr dimers have an extended four-helix coiled-coil signaling domain with a highly conserved hairpin tip (Figure 3). The tip promotes trimer formation and regulates CheA activity and is thought to play an important role in conformational transitions between the kinase-ON and kinase-OFF output states (Parkinson et al, 2015). The he mechanisms of signal transmission and kinase control by chemorecptors are largely unknown, but a phenylalanine (F) residue, at position 396 in Tsr plays a crucial role. This amino acid is the only universally conserved residue in MCP receptors (Ortega et al, 2013). An X-ray structure of Tsr showed that F396 residues interact across the subunit interface of the dimer tip (Figure 4; Kim et al, 1999). A subsequent all-atom molecular dynamics simulation of the Tsr dimer tip revealed that the F396/F396′ stacking interaction occasionally flipped and that the preferred stacking arrangement in a timer correlated with the receptor's adaptational modification state. An unmethylated Tsr model favored one arrangement; a highly methylated Tsr model favored the alternative stacking arrangement. Wild- type Tsr flipped between those two states, spending roughly equal time in each. These results suggested that the orientation of F396/F396′ stacking determines the Tsr signaling state and that a flip in the stacking interaction triggers a shift in Tsr output.
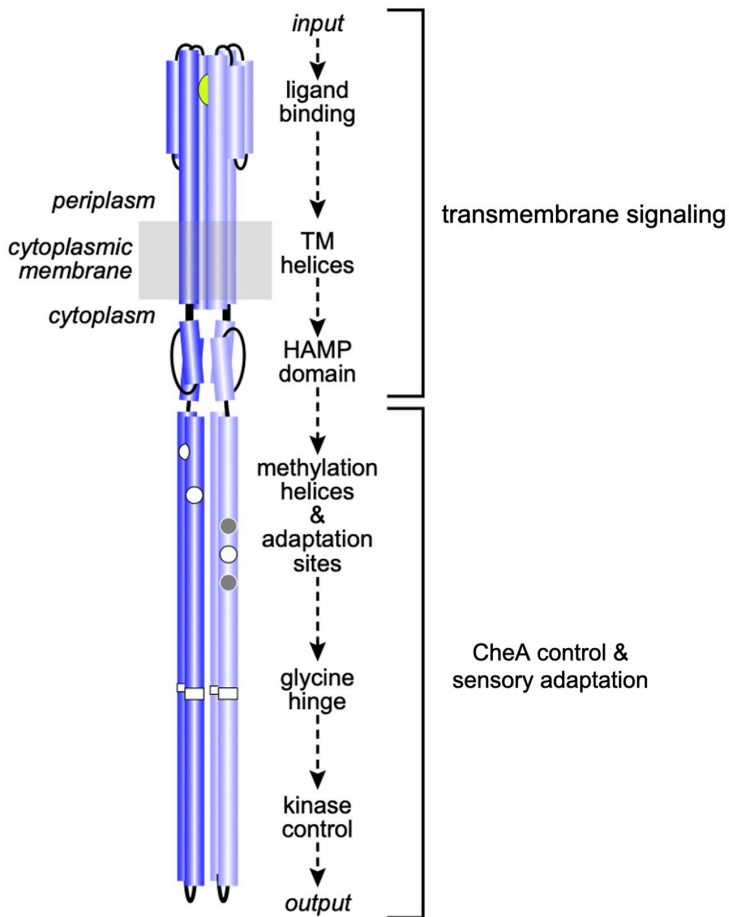
Figure 3: Signaling elements in the Tsr homodimer. Sensory information propagates from the ligand-binding domain to the kinase-control tip through structural interactions between relay elements.

To determine the importance of F396 to Tsr signaling, a series of Tsr mutants with all possible amino acid changes at residue 396 were constructed and assessed for chemotactic ability (Ortega et al, 2013). None of the mutant receptors could

support serine chemotaxis, indicating that phenylalanine at this position is indeed critical for proper receptor function. Subsequent studies of Tsr-F396* mutant receptors showed that many of them were strongly shifted toward a kinase-ON output state, suggesting that aromatic stacking of F396/F396′ might play an important role in stabilizing the kinase-OFF signaling state.

Interestingly, glycine (G), the smallest amino acid, and tryptophan (W), the largest, produced similar signaling defects when replacing the wild-type Tsr-F396 residue. Both the Tsr-

F396G and Tsr-F396W receptors were effectively locked in a kinase-ON output state in a cell lacking the sensory adaptation enzymes, CheR and CheB. But in an adaptation-competent host, both mutant receptors were able to shift to kinase-OFF output in response to a large serine stimulus. These results suggest that both the G and W side chains at Tsr residue 396 can support kinase-OFF signaling, but that output conformation may be much less stable than in the wild-type receptor. Conceivably, the inability of the mutant receptors to support chemotaxis, even in a cell with the adaption enzymes, is because they are too strongly biased toward the kinase-ON output state.
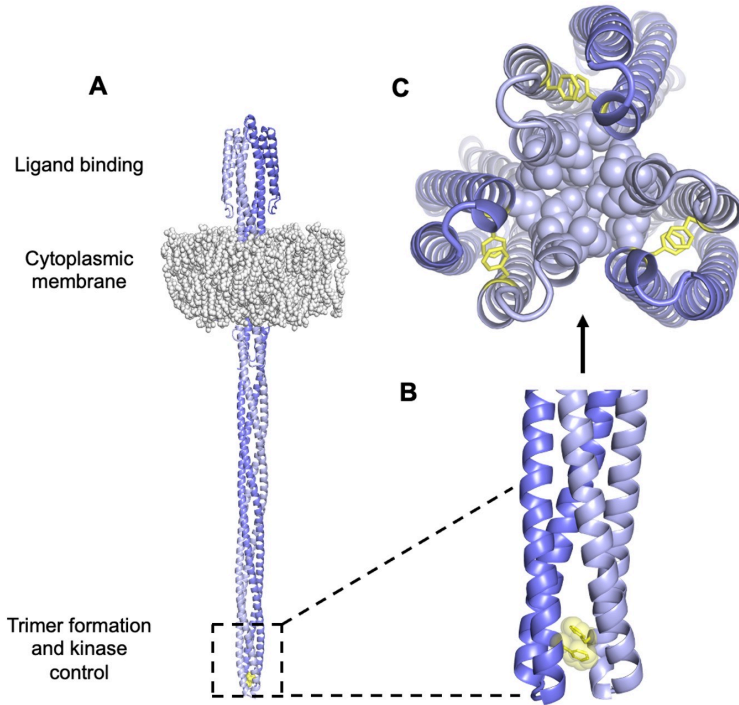
Figure 4: A. Model of Tsr. The hairpin tip circled in black holds amino acid residues that interact with other subunits to form trimer-of-dimers. Encompassed within this region is the conserved amino acid F396. B. These F396 residues lie at the interface between subunits of a dimer to interact in a specific conformation via stacking of its aromatic side chain. C. Tsr residues at the tip interact in trimer-of-dimers to stabilize the tip during kinase-on and kinase-off states.

My research employed reversion analysis of the Tsr-F396G and F396W receptors. I isolated and characterized a number of second-site mutations in the mutant *tsr* genes that could, to some extent, suppress their defective signaling properties. The signaling properties of the pseudorevertant (double mutant) receptors and of receptors carrying only the suppressor change

provided new mechanistic insights into the nature of hairpin tip signaling states and their control by adjacent signaling elements in the Tsr molecule. Moreover, this work has provided a new, testable model of how a chemoreceptor responds to sensory signals.

MATERIALS AND METHODS

Bacterial Strains

E. coli strains used were isogenic derivatives of K-12 RP437 (Parkinson et al, 1982). The relevant genotype of each strain used in subsequent experiments was: UU1623 [Δ*tap* Δ*tsr* Δ*trg* Δ*aer*]; UU1935 [*mutD5*]; UU2377 [*tsr*-R69E Δ(*tar-tap*) Δ*trg* Δ*aer*]; UU2378 [*tsr*-T156K Δ(*tar- tap*) Δ*trg* Δ*aer*]; UU2567 [Δ(*tar–cheZ*) Δ*tsr* Δ*aer* Δ*trg*]; UU2610 [Δ*aer* Δ(*tar*-cheB) Δ*tsr* Δ*trg*]; UU2612 [Δ*aer* Δ(*tar-tap*) Δ*tsr* Δ*trg*]; and UU3333 [Δ(*tar-tap*), Δ*tsr* Δ*trg* Δ*aer*] (Zhou et al, 2011; Lai et al, 2014).

Plasmids

Plasmids used in this project were: pKG116 (Gosink et al, 2006), which confers chloramphenicol resistance and has a salicylate-inducible expression/cloning site; pPA114, a pKG116 derivative that carries the wild-type *tsr* gene under salicylate-inducible control (Ames et al, 2002); pVS88, a plasmid compatible with pPA114 derivatives that confers ampicillin resistance and expresses the CheY-YFP and CheZ-CFP proteins under inducible IPTG control (Chang et al, 1978; Sourjik et al, 2007). IPTG is an analog of allolactose to act as an inducer for gene expression in the lac operon (Briand et al, 2016).

Growth media and buffers.

Cultures were grown in L broth (1% tryptone, 0.5% NaCl and 0.5% yeast extract) and colonies were harvested on L plates that contained L broths and 1.5% agar. Tryptone broth contained the same ingredients as L-broths but lacked the yeast extract. Solutions used in *in vivo* FRET based kinase assays included

KEP and Tethering buffer. KEP buffer contained 10 mM KPO4 (pH 7.0), and 0.1 mM K-EDTA. Tethering buffer was comprised of KEP with 10 mM Na- Lactate, 75 mM NaCl, 0.1 mM L-Methionine, and 100 ◇g/ml chloramphenicol (Parkinson et al, 1982).

Chemotaxis Assays

Serine chemotaxis ability of mutant strains was assessed on semi-solid tryptone agar plates that contained 12.5 μg/ml chloramphenicol and 0.6 μM sodium salicylate (Parkinson, 1980). These plates permit the bacteria to swim in a semi-liquid media. Plates were incubated at 32.5℃ for 6 to 8 hours. Mutants were tested for temperature-dependent defects at 25℃ and 37℃.

Isolation of chemotactic pseudorevertants

Mutant *tsr* genes were manipulated and expressed in derivatives of pPA114. Tsr-F396W and Tsr-F396G) plasmids were subjected to random mutagenesis by replication in UU1935.

Plasmid pools from UU1935 were introduced UU2612, and 50 μl aliquots of the transformation mix were streaked across T-swim plates to reveal chemotactic clones after incubation at 32.5℃ for 16 hours (Ames et al, 2002). Plasmids were isolated from revertants using QIAGEN DNA extraction kits. Their mutant *tsr* genes were sequenced by the Protein-DNA Core Facility of the University of Utah.

Expression levels of Mutant Tsr Proteins

Strain UU2610 (CheR-, CheB-) was used as a host to assess Tsr protein expression levels from mutant plasmid derivatives. Cells were grown in tryptone broth with appropriate antibiotics to the mid-exponential phase. Cells were washed, resuspended in Laemmli sample buffer, and lysed by boiling for five minutes (Laemmli, 1970). Samples were analyzed by

electrophoresis in denaturing polyacrylamide gels, and Tsr bands were visualized by immunoblotting with a polyclonal rabbit antiserum directed against the Tsr cytoplasmic domain (Mowery et al, 2002; Ames et al, 1994). Tsr bands were quantified using ImageJ software.

Dominance and Jamming Assays

Dominance assays of suppressor mutants were performed in strains UU2377 and UU2378 as described by Ames et al. Jamming assays of suppressor mutants were performed in strain UU1623 as described by Ames et al.

In vivo FRET-based Kinase Assays

The protocol and data analysis for *in vivo* FRET-based kinase assays followed the methods described by (Sourjik et al, 2007). Mutant Tsr plasmids and the FRET reporter plasmid, pVS88were introduced into strain UU2567. The transformant cells were grown at 30℃ for five hours to mid-exponential phase in tryptone broth (50 μg/ml ampicillin, 12.5 μg/ml chloramphenicol, 100 μg/ml sodium salicylate, IPTG). Cells, were washed by centrifugation, bound to a polylysine-treated cover slip, and mounted in a flow cell maintained at 30℃ throughout each experiment). Cells were subjected to a series of serine concentrations and the dose-response data were analyzed with Kaleidagraph software. In the absence of a serine response, receptor-generated kinase activity was measured by the FRET change elicited by 3 mM KCN (Lai & Parkinson, 2014).

RESULTS

Pseudoreversion analysis of Tsr-F396G and Tsr-F396W mutant chemoreceptors

To determine whether it is possible to "heal" the functional

defects of the Tsr-F396G and Tsr-F396W receptors, I looked for second-site mutations within the mutant *tsr* coding sequence that could suppress their defects (Figure 5). Mutant receptor genes carried in plasmid pPA114 derivatives were mutagenized in an error-prone *mutD* host. Treated plasmids were transformed into recptorless host UU2612 and chemotactic pseudorevertants were picked from soft agar plates. These chemotactic pseudorevertants presumably contained suppressor mutations that improved the function of the parental mutant receptor (Figure 5). I then identified the suppressor mutations in the pseudorevertant plasmids by DNA sequence analysis in the *tsr* coding region.

To isolate the suppressor mutants from the double mutants, I converted the Tsr-F396G and Tsr- F396W mutation in the pseudorevertants back to wild-type (F396) using sequence-targeted PCR. I obtained 3 different suppressor mutations for F396W, and 16 different suppressors for F396G (Figure 6A). All of these mutations were single base pair substitutions. With one exception (D481N), their inferred amino acid replacements fell within the hairpin tip or flexible bundle of Tsr. Second-site suppressors of F369G produced more robust chemotaxis than those of F396W, with several F369G suppressors performing as well as wild-type Tsr on soft agar swim plates (See Appendix; Table 1).

Among Tsr-F396G pseudorevertants, the S327N suppressor was found four times; A323V, D363G, and I455V were each found twice. For Tsr-F396W, A382V and A382T were each obtained twice. Overall, I found an average of about 1.4 mutations per suppressor site, which, if all mutations arose independently, indicates that the mutant hunt was not fully saturated and that there may be additional suppressors yet undiscovered.

Chemotaxis promoted by the mutant chemoreceptors

I tested the serine chemotaxis phenotypes of the doubly-mutant pseudorevertant receptors in soft agar plates (see example in Figure 6B). In the UU2612 host, all F396G pseudorevertant receptors produced a phenotype similar to wild-type Tsr. The double mutant Tsr- F396G/V466T supported chemotaxis better than the wild-type Tsr. The F396W pseudorevertant receptors exhibited only partial chemotactic function. The Tsr-F396W defect is evidently difficult or impossible to fully suppress, in contrast to that of Tsr-F396G.
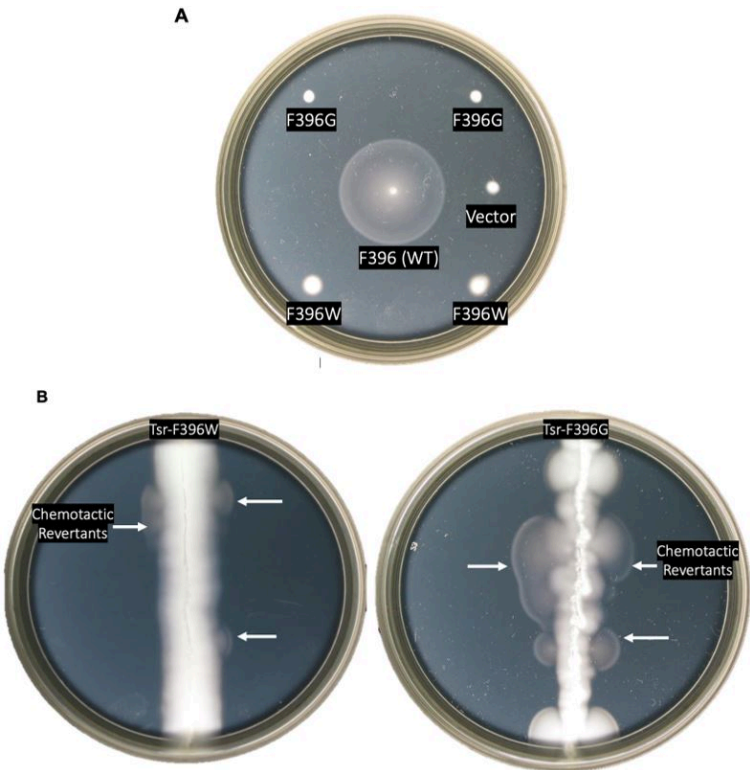
Figure 5: Chemotaxis assays of mutant receptors. A. Chemotactic behaviors of Tsr- F396G and Tsr-F396W mutants

as compared to F396 wild-type on soft agar plates. The strains that contained wild-type, mutant, or vector plasmids were all screened for chemotaxis. B. Screening for serine chemotaxis after random mutagenesis in a host that
is Δmcp. White arrows represent swarms containing chemotactic pseudorevertants.

Chemotaxis promoted by the mutant chemoreceptors

I tested the serine chemotaxis phenotypes of the doubly-mutant pseudorevertant receptors in soft agar plates (see example in Figure 6B). In the UU2612 host, all F396G pseudorevertant receptors produced a phenotype similar to wild-type Tsr. The double mutant Tsr- F396G/V466T supported chemotaxis better than the wild-type Tsr. The F396W pseudorevertant receptors exhibited only partial chemotactic function. The Tsr-F396W defect is evidently difficult or impossible to fully suppress, in contrast to that of Tsr-F396G.

To characterize the functional properties of receptors carrying only a suppressor change, I converted the F396 mutant codon in each pseduorevertant back to wild type (F396) using sequence-targeted PCR. In host strain UU2612, 9 out of 17 of the single-mutant receptors exhibited wild-type Tsr function on soft agar plates, 4 out of 17 had partial function, and 4/17 were nonfunctional (See Appendix). None of the suppressors displayed chemotactic function greater than the double-mutants, *i.e.* F396* plus the second-site mutation. Additional functional tests to determine whether these mutant receptors are capable of making stable protein and characterizing their signaling properties with FRET kinase assays will reveal structural features important for receptor signaling.
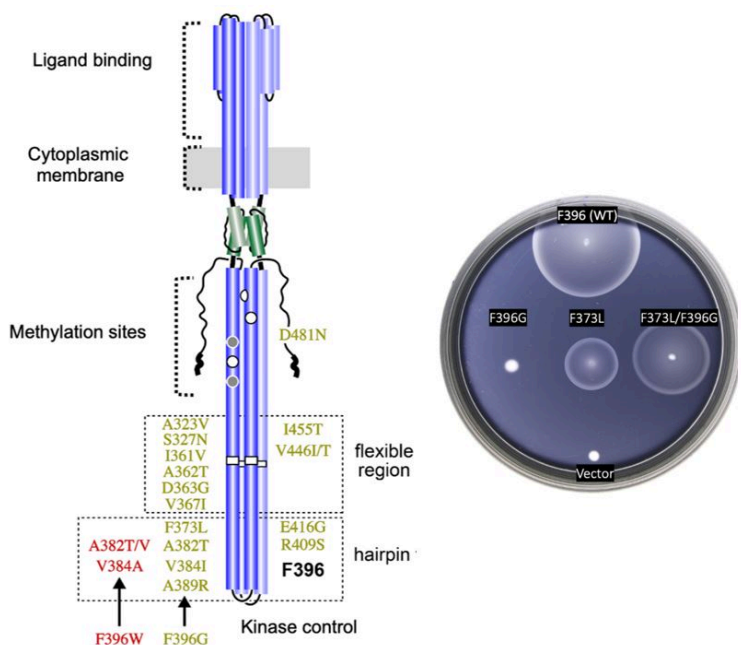
Figure 6: Found suppressor mutants in *tsr*. A. Summary of the glycine (green) and tryptophan (red) suppressors found within the Tsr receptor. B. Example of chemotactic behavior of the original mutant F396G (left), the isolated suppressor mutant (center), and the double mutant

(right).

Immunoblot Analysis of Mutant Receptors

I measured the intracellular levels of the pseudorevertant and suppressor proteins by gel electrophoresis and anti-Tsr immunoblotting of cell extracts. All mutant proteins had steady-state levels comparable to wild-type Tsr (Figure 8). This result is particularly important for the receptors that failed to support chemotaxis because it means that the mutant protein is expressed normally but cannot function normally.

Signaling Properties of Mutant Tsr Proteins

To elucidate the functional properties of my mutant

receptors, I used a FRET-based kinase assay {Sourjik et al, 2002) to determine the ability of the mutant receptors to modulate CheA activity in response to serine stimuli. I expressed the mutant receptors in UU2567, a host that lacks the sensory adaptation enzymes CheR and CheB. In this reporter strain, wild-type Tsr molecule produces relatively high kinase activity that responds to serine stimuli with half- maximal inhibition ($K1/2$) at ~18 μM (Figure 9A). The signaling behaviors of the mutant receptors were interpreted in the context of a two-state model of kinase-OFF and kinase-ON receptor output states (see Figure 2). Off-shifted mutant receptors have higher serine response sensitivity (lower

$K1/2$), whereas ON-shifted mutant receptors reduce serine response sensitivity and show higher

$K1/2$) values or fail to respond at even very high serine concentrations.
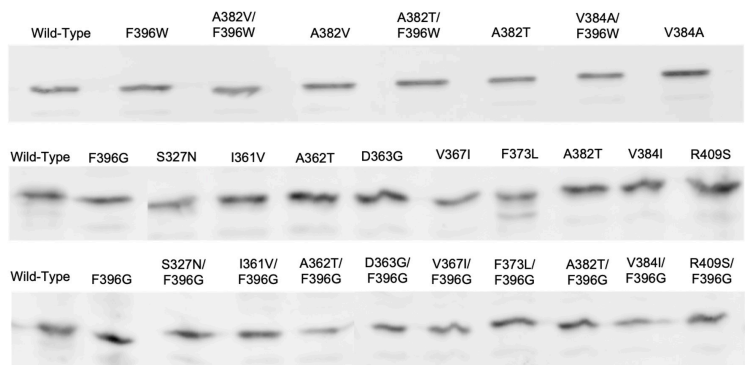
Figure 8: Immunoblots of Tsr-F396G/W double mutants and suppressors. Protein expression samples were expressed in strain UU2610, a host which lacks sensory adaptation enzymes

CheR and CheB. Wild-type Tsr is expressed in the far-left column.

Tsr-F396G exhibited no response to serine in the UU2567 host but did evince some kinase activity when treated with KCN (Figure 9B). This behavior indicates that F396G has a non-responsive kinase-ON output state. Similarly, Tsr-F396W failed to respond to any level of serine concentration but produced kinase-ON output (Figure 9B).

Note that both Tsr-F396* mutant receptors only produced about 50% of the Tsr wild-type kinase activity. This is an important clue to the possible mechanistic basis of their signaling defects, as detailed in the Discussion. A majority (12 out of 17) of the receptors carrying suppressor changes alone also exhibited non-responsive-ON behavior (Figure 10). Two suppressor mutants exhibited locked-OFF outputs, and three responded to serine with moderate sensitivities, but very low cooperativities (Hill coefficients <5; wild-type Hill ~15).

Signaling Properties of Pseudorevertant Receptors

I utilized FRET-based kinase CheA assays to evaluate the signaling properties of the Tsr- F396G/W pseudorevertant receptors. In the host UU2567 (CheR-, CheB-), the F396G double mutants with suppressor changes in the flexible bundle region of Tsr were capable of modulating CheA activity in response to serine stimuli but had K1/2 values above that of wild-type Tsr, indicative of ON-shifted behavior (Figure 11).

In UU2567, F396G pseudorevertants with suppressor changes in the hairpin tip region were unresponsive to serine stimuli but exhibited kinase activity in the KCN assay (Figure 11). This behavior indicates a strong shift non-responsive shift toward a kinase-ON output state. It is important to note that these response data were collected in a host strain that lacked the adaptation enzymes (CheR-, CheB-). However, the doubly

mutant receptors can support chemotaxis in an adaptation-competent (CheR+ CheB+) strain.

These results indicate that there may be more than one kinase-ON output state that enables these mutant receptors to be shifted toward the kinase-OFF output state. Continued work in the FRET reporter strain UU2700 will further elucidate the mechanism behind this model, as summarized in the Discussion.
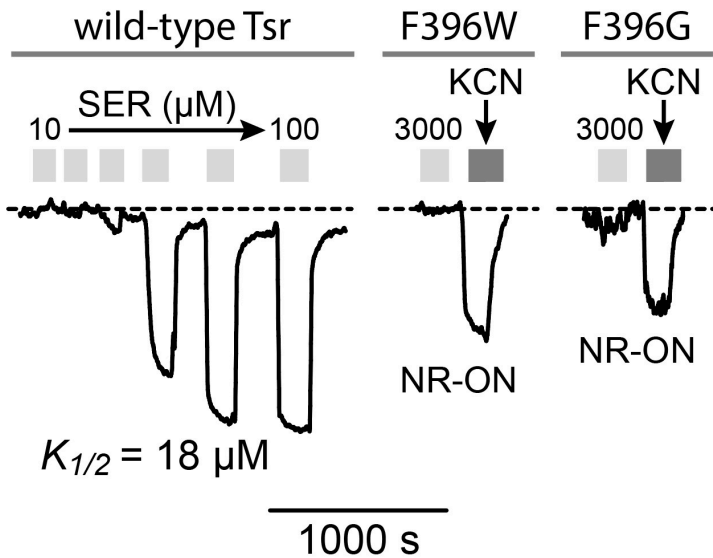
Figure 9: FRET kinase readouts. A. Example of wild-type Tsr FRET kinase readout. The y- axis represents the FRET signal which directly measures kinase activity (Sourjik et al, 2007). Light grey bars represent each time the receptor was introduced to serine. The wild-type receptor responds to serine and inhibits kinase activity. B. Dark grey bars represent kinase activity. Apparent kinase activity of mutant receptors F396G and F396W is not as high as the wild-type receptor.
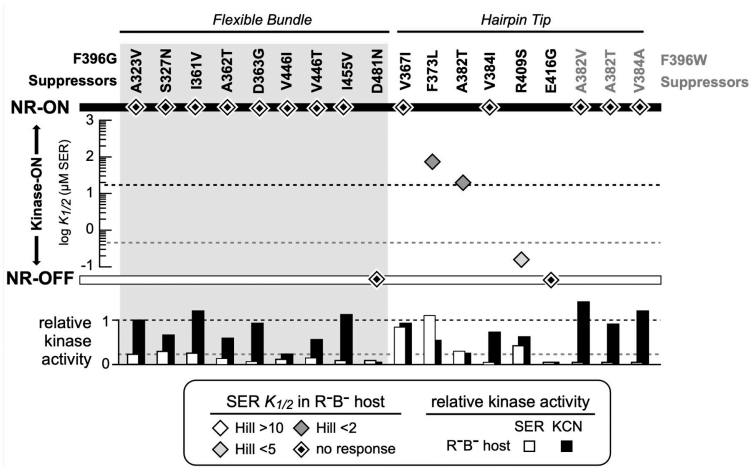
Figure 10: FRET Kinase assay of suppressor mutants for both Tsr-F396G and Tsr- F396W. The diamonds indicate the K1/2 measurement in the host UU2567 (CheR-, CheB-). Suppressor mutants that produce kinase activity but are unresponsive to serine are indicated by a diamond on the NR-ON bar. Suppressor mutants that are both unresponsive to serine and do not produce kinase activity are indicated by a diamond on the NR-OFF bar. The bottom graph depicts the relative kinase activity of the suppressor mutants in UU2567.
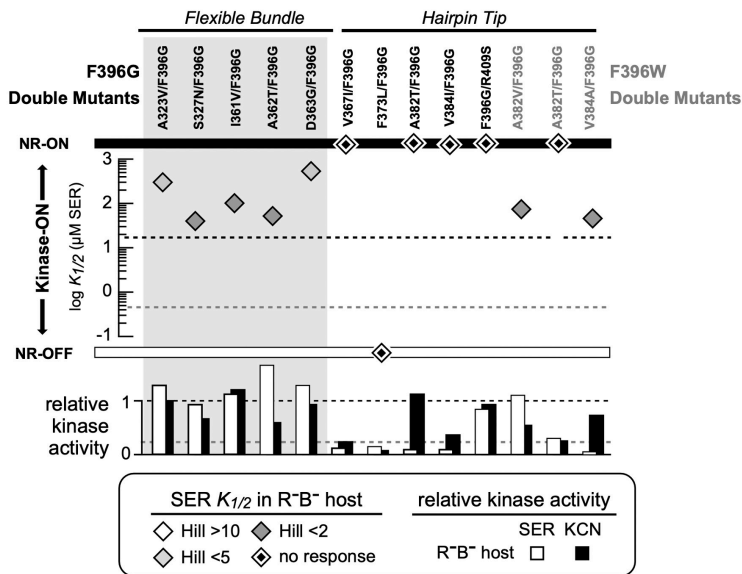
Figure 11: FRET Kinase assay of double mutants for both Tsr-F396G and Tsr-F396W mutants. The diamonds indicate the K1/2 measurement in the host UU2567 (CheR-, CheB-). Double mutants that produce kinase activity but are unresponsive to serine are indicated by a diamond on the NR-ON bar. Suppressor mutants that are both unresponsive to serine and do not produce kinase activity are indicated by a diamond on the NR-OFF bar. The bottom graph depicts the relative kinase activity of the suppressor mutants in UU2567.

DISCUSSION

In this project I isolated and characterized second-site mutations that effectively healed the Tsr-F396G and Tsr-F396W signaling defects. In the following, I will discuss these results in the context of a two-state receptor signaling model and suggest a suppression mechanism that involves dynamic signaling states at the cytoplasmic tip of the receptor. Moreover, the investigation of this position will uncover the mechanisms by

which Tsr generates its locomotor- controlling signals and, in part, answer the question of how signaling is transmitted throughout the receptor–a pathway that is universal across organisms.

F396 as a switch for signal-state control

The current view of protein allostery hypothesizes that chemoreceptors such as Tsr occupy different conformations to modulate signal outputs. Inputs such as serine change the conformational landscape of the receptor to shift the hairpin tip to a kinase-OFF conformation (Ortega et al, 2013). In the absence of a chemical gradient, the Tsr receptor is mainly in a kinase- ON state.

F396 appears to play a central role in the ON-OFF conformational switch through its aromatic stacking interactions. One F396/F396' stacking arrangement produces kinase-ON output; a flip in their stacking arrangement promotes an alternative interaction to shift Tsr to the kinase- OFF state (Ortega et al, 2013). This interaction occurs synchronously with signal conformations at the tip of the receptor. No other amino acid residue at Tsr residue 396 is able to promote wild-type chemotaxis. Many of such mutants are stuck in kinase-ON output, indicating that F396 is important for stabilizing the kinase-OFF confirmation of the receptor.

Suppression of F396 mutant signaling behaviors

To elucidate the mechanistic role that F396 plays, I hypothesized that second-site suppressors of F396G and F396W might act by enabling them to once again access the kinase- OFF state in response to serine stimuli. Six F396G suppressors and all

three F396W suppressors altered hairpin tip residues (Figure 6). Of these, two F396G suppressors (F373L and V384I) were at trimer contact residues These results indicate that compensatory changes in the hairpin tip may enhance stability of the kinase-OFF output state. Nine F396G suppressors altered residues in the flexible region that adjoins the hairpin tip. These suppressor changes may alter the transmission of stimulus-induced conformational signals that modulate the structure of the hairpin tip.

The signaling properties of the parental F396G and F396W mutant receptors indicate that they might affect Tsr performance differently. I theorized that the F396W defect would be easier to heal because its aromatic side chain is very similar, albeit larger, than that of the wild-type phenylalanine. Interestingly, this was not the case. F396G pseudorevertants arose more readily and regained better chemotactic ability than did F396W double mutants. Perhaps the size of the amino acid sidechain at residue 396 is an important factor in its function. Although Tsr-F396W has an aromatic side chain similar to that of phenylalanine, its larger size might actively perturb the structure of the hairpin tip. Or, more likely, its large size makes switching between kinase-ON and kinase-OFF output states more difficult due steric hindrance.

Paradoxical signaling properties of Tsr mutant receptors

In the host UU2567, Tsr-F396G and Tsr-F396W failed to respond to any concentration of serine but were able to promote kinase activity (Figure 9). However, their kinase activity was only about half that of the native receptor. I expected the their suppressors might promote a kinase- OFF signaling behavior and thereby "offset" the locked-ON defects of the parental F396 mutant receptors.

Surprisingly, most of the suppressor changes created ON-shifted or locked-ON output behaviors. How might F396G (NR-ON) and its suppressors (NR-ON) create a double mutant capable of modulating serine? Perhaps there is a range of chemoreceptor conformations at the hairpin tip that elicit kinase activity over a number of dynamic states. Thus, there may exist more than one receptor ON state that can be driven toward a kinase-OFF output state by an attractant stimulus.

Operational conformations at the tip of the receptor

I present my mechanistic ideas in the context of a model where the receptor tip of Tsr can transition across a structural landscape characterized by different conformations and different dynamic behaviors. I propose that the receptor and its signaling hairpin tip operate across a variety of landscapes that define the kinase-ON and kinase-OFF output states (Figure 12A). As the receptor traverses these landscapes, its kinase activity will fluctuate. As such, the ON state of a mutant

receptor might not be identical to the level of kinase activity in a wild-type receptor.

Suppose that the wild-type receptor (Tsr) operates within the shaded region of the conformational landscape shown in Figure 12B. In this regime, the attractant can interact with a receptor that is in the kinase-ON output state to drive it to kinase-OFF. Conversely, in the absence of the attractant, the native receptor will fluctuate between kinase-ON and kinase-OFF regions of the landscape. It is important to note that Tsr operates over a range of conformations that are conducive to control by the adaptation enzymes. The enzyme CheR operates on OFF-state receptors to shift them toward ON output by adding methyl groups to the receptor adaptation sites. Alternatively, the demethylating enzyme CheB operates on kinase-ON receptors and shifts them toward the OFF output state. In a two-state equilibrium the wild-type receptor can access both output states. However, if CheB is unable to operate on ON-state receptors that are too far outside of the normal wild-type range this would suggest that a two-state model of receptor signaling is too simplistic.
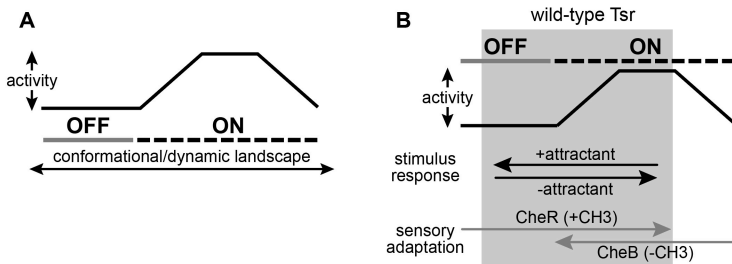
Figure 12: A graphical representation of the two-state model with dynamic "on" states. A. There may exist an operational landscape over which Tsr can transition by different

conformations and differ dynamic behavior at the cytoplasmic tip which define the output states of kinase-on and kinase-off. B. Wild-type Tsr is works in the grey shaded region of the landscape and can shift between kinase-on and kinase-off states.

Figure 13 shows the conformational landscape of the Tsr-F396G receptor. As previously mentioned, F396G is constrained to a conformational landscape that produces less kinase activity than the native receptor and that cannot be driven OFF by serine (Ortega et al, 2013). To restore serine responsiveness, F396G must acquire a kinase-ON conformation similar to that of wild- type (Figure 13B).

I hypothesize that a suppressor of the F396G defect might itself be off-shifted and thus capable of shifting the doubly mutant receptor into a more wild-type conformational landscape. However, FRET kinase assays revealed that many of the found suppressor mutants did not respond to serine and produced less than the wild-type kinase activity. It seems as though the suppressor changes have constrained the kinase-ON conformational range of the receptor so that it is no longer capable of reaching the off-state. By contrast, in a low modification state, for example through CheB action, the Tsr-F396G can reach the serine-induced OFF state. Perhaps in combination, the F396 and suppressor defects interact in a way that shifts the conformational landscape of the doubly mutant receptor toward the wild-type operational range.

Evidence for different operational landscapes in different output states

Surprisingly, this interaction is both necessary and sufficient for proper transmembrane signing by Tsr pseudorevertant receptors. The conformational range of the doubly mutant receptor is much wider than that of the suppressor or of F396G

separately. This could occur through two conformational interactions: one that "pulls" the F396G defect into a higher kinase activity range similar to that of wild-type Tsr, and one that confers serine-responsiveness to the suppressor defect. Thus, together the two defects create a receptor whose operational landscape is similar to the wild-type receptor and that supports chemotaxis.

A

Tsr-F396G

OFF   **ON**

activity

stimulus
response

+attractant

-attractant

sensory
adaptation

CheR (+CH3)

CheB (-CH3)

B

Tsr-sup

OFF   **ON**

activity

stimulus
response

+attractant

-attractant

sensory
adaptation

CheR (+CH3)

CheB (-CH3)

C

Tsr-F396G/sup*

OFF   **ON**

activity

stimulus

+attractant
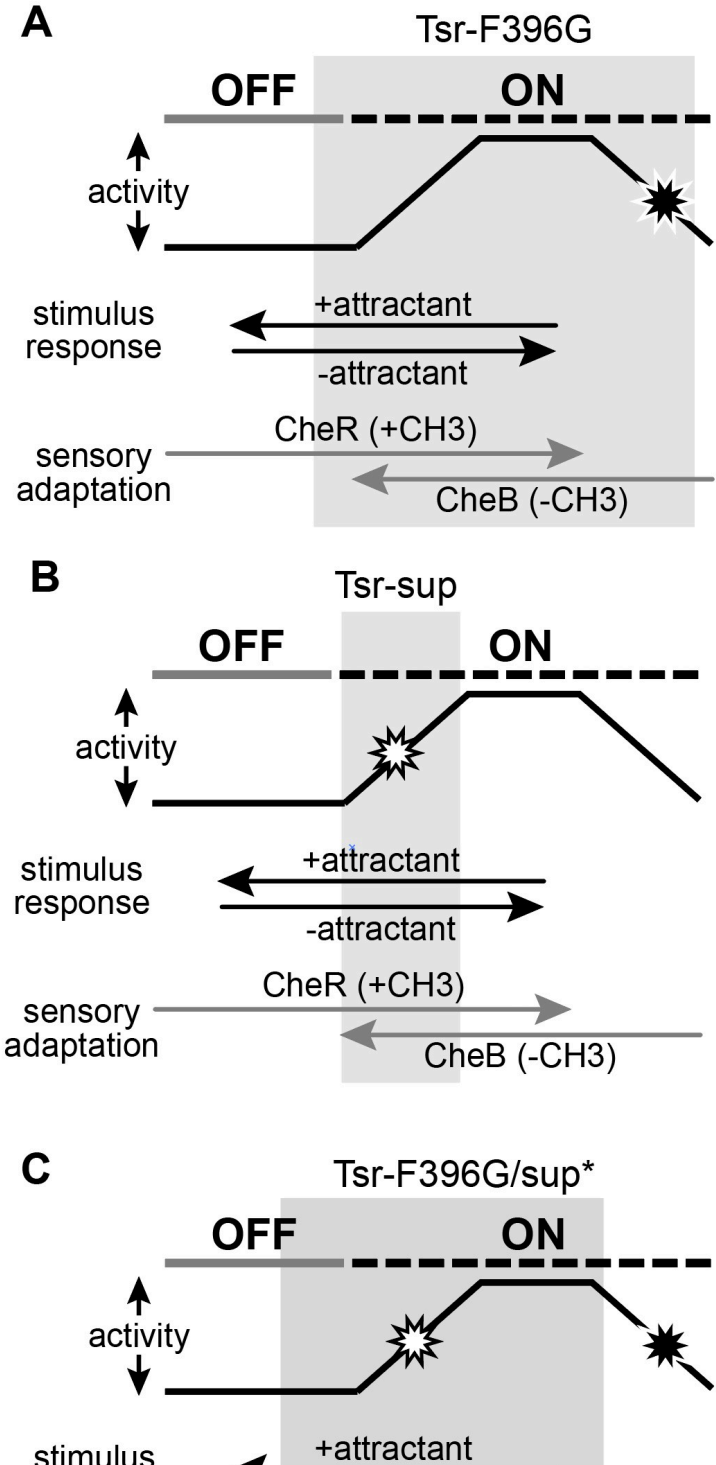
Figure 13: Conformational landscapes of mutant receptors. A. Tsr-F396G mutant receptor operates in the grey region of the landscape. It is not capable of reaching the kinase-off state without the adaptation enzyme CheB. B. Suppressor mutants have constricted the conformation range over which the receptor can operate, as shown by the grey bar. C. Double mutants have increased the conformational range over which the receptor can access both kinase-on and kinase-off states, shifting it into a landscape similar to the wild-type.

REFERENCES

Ames P, Parkinson JS. 1994. Constitutively signaling fragments of Tsr, the *Escherichia coli* serine receptor. J Bacteriol **176:**6340-6248.

Ames P, Parkinson JS. Conformational suppression of inter-receptor signaling defects. Proc Natl Acad Sci U S A. 2006 Jun 13;103(24):9292-7. doi: 10.1073/pnas.0602135103. Epub 2006 Jun 2.

PMID: 16751275; PMCID: PMC1482603.

Ames P, Studdert CA, Reiser RH, Parkinson JS. 2002. Collaborative signaling by mixed chemoreceptor teams in *Escherichia coli.* Proc Natl Acad Sci USA **99:**7060-7065. http://dx.doi.org/10.1073/pnas.092071899.

Ames, Peter, et al. "Collaborative Signaling by Mixed Chemoreceptor Teams in Escherichia Coli." *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 10, 2002, pp. 7060–65. *JSTOR*, http://www.jstor.org/stable/3058791. Accessed 30 Nov. 2022.

Ariane Briegel, Mark S Ladinsky, Catherine Oikonomou, Christopher W Jones, Michael J Harris, Daniel J Fowler, Yi-Wei Chang, Lynmarie K Thompson, Judith P Armitage, Grant J

Jensen (2014) Structure of bacterial cytoplasmic chemoreceptor arrays and implications for chemotactic signaling eLife 3:e02151 https://doi.org/10.7554/eLife.02151

Bi S, Lai L. Bacterial chemoreceptors and chemoeffectors. Cell Mol Life Sci. 2015, Feb;72(4):691-708. doi: 10.1007/s00018-014-1770-5. Epub 2014 Nov 6. Review. PubMed PMID: 25374297.

Briand L, Marcion G, Kriznik A, Heydel JM, Artur Y, Garrido C, Seigneuric R, Neiers F. A self- inducible heterologous protein expression system in Escherichia coli. Sci Rep. 2016 Sep 9;6:33037. doi: 10.1038/srep33037. PMID: 27611846; PMCID: PMC5017159.

Chang ACY, Cohen SN. 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic miniplamid. J Bacteriol **134:**1141-1156

Kitanovic S, Ames P, Parkinson JS. A Trigger Residue for Transmembrane Signaling in the Escherichia coli Serine Chemoreceptor. J Bacteriol. 2015 Aug 1;197(15):2568-79. doi: 10.1128/JB.00274-15. Epub 2015 May 26. PMID: 26013490; PMCID: PMC4518824.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4.
Nature. 1970 Aug 15;227(5259):680-5. doi: 10.1038/227680a0. PMID: 5432063.

Lai RZ, Han XS, Dahlquist FW, Parkinson JS. Paradoxical enhancement of chemoreceptor detection sensitivity by a sensory adaptation enzyme. Proc Natl Acad Sci U S A. 2017

Sep 5;114(36):E7583-E7591. doi: 10.1073/pnas.1709075114. Epub 2017 Aug 21. PMID: 28827352; PMCID: PMC5594695.

Lai RZ, Parkinson JS. 2014. Functional suppression of HAMP domain signaling defects in the *E. coli*

serine   chemoreceptor.   J   Mol   Biol   **426:**3642-3655 http://dx.doi.org/10.1016/j.jmb.2014.08.003.

Ortega, Davi R et al. "A phenylalanine rotameric switch for signal-state control in bacterial chemoreceptors." *Nature communications* vol. 4 (2013): 2881. doi:10.1038/ncomms3881

Parkinson JS, Hazelbauer GL, Falke JJ. Signaling and sensory adaptation in Escherichia coli chemoreceptors: 2015 update. Trends   Microbiol.   2015   May;23(5):257-66.   doi: 10.1016/ j.tim.2015.03.003. Epub 2015 Mar 30. PMID: 25834953; PMCID: PMC4417406.

Parkinson JS, Houts SE. 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. J Bacteriol **151:**106-113

Paulick A, Sourjik V. FRET Analysis of the Chemotaxis Pathway Response. Methods Mol Biol.

2018;1729:107-126. doi: 10.1007/978-1-4939-7577-8_11. PMID: 29429087.

Piñas G.E., DeSantis M.D., Cassidy C.K. and J.S. Parkinson (2022) Hexameric rings of the scaffolding protein CheW enhance response sensitivity and cooperativity in *Escherichia*

coli chemoreceptor arrays. *Sci. Signal.*, **15**: DOI: 10.1126/scisignal.abj1737.

Piñas GE, DeSantis MD, Cassidy CK, Parkinson JS. Hexameric rings of the scaffolding protein CheW enhance response sensitivity and cooperativity in *Escherichia coli* chemoreceptor arrays. Sci Signal. 2022 Jan 25;15(718):eabj1737. doi: 10.1126/scisignal.abj1737. Epub 2022 Jan 25. PMID: 35077199; PMCID: PMC9261748.

Silversmith RE, Guanga GP, Betts L, Chu C, Zhao R, Bourret RB. CheZ-mediated dephosphorylation of the Escherichia coli chemotaxis response regulator CheY: role for CheY glutamate 89. J Bacteriol. 2003 Mar;185(5):1495-502. doi: 10.1128/JB.185.5.1495-1502.2003. PMID: 12591865; PMCID: PMC148069.

Sourjik V, Berg HC. 2002. Receptor sensitivity in bacterial chemotaxis. Proc Natl Acad Sci U S A

99:123-127. http://dx.doi.org/10.1038/nature02406.

Sourjik V, Vaknin A, Shimizu TS, Berg HC. 2007. In vivo measurement by FRET of pathway activity in bacterial chemotaxis. Methods Enzymol **423:**365-391. http://dx.doi.org/10.1016/S0076- 6879(07)23017-4.

Stalla D, Akkaladevi N, White TA, Hazelbauer GL. Spatial Restrictions in Chemotaxis Signaling Arrays: A Role for Chemoreceptor Flexible Hinges across Bacterial Diversity. Int J Mol Sci. 2019 Jun 19;20(12):2989. doi: 10.3390/ijms20122989. PMID: 31248079; PMCID: PMC6628036.

Yang W, Cassidy CK, Ames P, Diebolder CA, Schulten K, Luthey-Schulten Z, Parkinson JS, Briegel A. *In Situ* Conformational Changes of the Escherichia coli Serine Chemoreceptor in Different Signaling States. mBio. 2019 Jul 2;10(4):e00973-19. doi: 10.1128/mBio.00973-19. PMID: 31266867; PMCID: PMC6606802.

Zhou Q, Ames P, Parkinson JS. 2011. Biphasic control logic of HAMP domain signaling in the Escherichia coli serine chemoreceptor, Mol Microbiol **80:**596-611. http://dx.doi.org/10.1111/j.1365-2958.2011.07577.x.

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110.   Stability of Genetic Oscillators with Distributed Delayed Feedback

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ABSTRACT

Genetic oscillators govern periodic phenomena in biology including circadian rhythms and are also the basis of biological clocks used in the design of synthetic genetic circuits. Models of genetic oscillators tend to neglect biological detail, however, because biological systems tend to be too complicated to model efficiently. One way to incorporate additional biological detail into models of genetic oscillators is to use distributed delay differential equations. To investigate the utility of distributed delay differential equations for modeling genetic oscillators, we constructed delayed differential equation models of genetic

oscilla- tory motifs. We found that these models are equivalent to higher-dimension models, which are reflective of more granular biological detail. We also characterized the stability of these models. Our findings may inform future modeling efforts in the domains of synthetic and systems biology, where delayed differential equations could pose advantages over ordinary differential equation models.

INTRODUCTION

Genetic oscillators, which are intrinsic time-keeping devices in living organisms, have been the subject of research in the field of mathematical biology for some time [1, 2]. The study of these oscillators has led to a deeper understanding of the molecular mechanisms that underlie a wide range of biological processes, including circadian rhythms [3], cell cycle regulation [4], and developmental processes [5]. Circadian rhythms, for instance, are endogenous rhythms that persist in the absence of external cues and are responsible for co- ordinating a wide range of physiological and behavioral processes [6]. Biological clocks, based on the principles of genetic oscillators, have been engineered to regulate the expres- sion of genes in synthetic circuits [7, 8], with applications ranging from biosensors [9] to drug delivery systems [10].

While genetic oscillators have become increasingly well understood, modeling their behavior presents a significant challenge [11]. Mathematical models of genetic oscilla- tors must balance the need for accuracy with computational efficiency, as the large number of molecular

interactions involved in these systems can be computationally expensive to model in detail [12]. As a result, models of genetic oscillators tend to simplify the un- derlying biology, often neglecting important biological details in favor of simplicity and efficiency [13, 14]. These simplified models may fail to reflect dynamics observed *in vivo* by, for example, altering system stability [11].

Distributed delay differential equations are a promising approach to modeling genetic oscillators with greater biological detail [15]. Unlike ordinary differential equations which ignore time delays entirely and discrete delay differential equations which assume constant delay in feedback steps [16, 17], distributed delay differential equations allow for a distri- bution of delays that more closely approximates the complex biological interactions that occur in living organisms. These models have been shown to accurately capture the be- havior of some simple genetic oscillators [18], while also remaining intuitive and simple to simulate. To investigate the utility of distributed delay differential equations for model- ing genetic oscillators, we constructed and analyzed distributed delay differential equation models of repressilators, one important class of synthetic genetic oscillator [2, 7].

BACKGROUND

A   Distributed Delays

Delay differential equations (DDEs) are a class of functional differential equations wherein the dynamics of a system depend on the past state(s) of the system as well as its current state. Unlike ordinary differential equations (ODEs), DDEs are infinite dimensional in phase space [19, 20]. In general, DDEs may be written as

$$\frac{d}{dt}x(t) = f(t, x(t), x_t), \quad \text{where } x_t = \{x(\tau) : \tau < t\}. \tag{1}$$

The simplest DDEs are *discrete DDEs*: a subset of DDEs wherein only discrete, constant time delays are present [21]. Discrete DDEs may be written as

$$\frac{d}{dt}x(t) = f(t, x(t), x(t-\tau_1), x(t-\tau_2), \ldots, x(t-\tau_n), \quad \text{where } \tau_1 > \tau_2 > \ldots \tau_n > 0. \tag{2}$$

In systems where time delay is uncertain or variable, it may be more appropriate to use *DDEs with random delays* [21]. Such systems may be written as

$$\frac{d}{dt}x(t) = f(t, x(t), x(t-\Delta_1), x(t-\Delta_2), \ldots, x(t-\Delta_n)), \tag{3}$$

where each of $\Delta_1, \ldots, \Delta_n$ is a non-negative random variable. A DDE with random delays may be approximated as the deterministic *distributed DDE* given by its expectation [21]:

$$\frac{d}{dt}x(t) = f(t, x(t), \mathbb{E}[x(t-\Delta_1)], \mathbb{E}[x(t-\Delta_2)], \ldots, \mathbb{E}[x(t-\Delta_n)]), \tag{4}$$

where $\mathbb{E}$ is the expectation operator. For example, the simple DDE with random delays given by

$$\frac{d}{dt}x(t) = \alpha x(t-\Delta) \tag{5}$$

may be approximated as

$$\frac{d}{dt}x(t) = \alpha \mathbb{E}[x(t-\Delta)] = \alpha \int_0^\infty x(t-\tau) f_\Delta(\tau) d\tau, \tag{6}$$

where $f_\Delta(\cdot)$ is the *probability density function* (PDF) of Δ. This demonstrates that distributed DDEs are a class of *integro-differential equations* (IDEs).

DDEs may sometimes be reduced to systems of ODEs [22]. To illustrate this, consider the case when Δ is distributed according to an exponential distribution. In this case we may reduce as follows,

$$\frac{d}{dt}x(t) = f\left(t, x, \int_{-\infty}^0 x(t+\tau) e^{\lambda \tau} d\tau\right) \tag{7}$$

Let $y(t) = \int_{\infty}^{0} x(t+\tau)e^{\lambda\tau}d\tau = \int_{-\infty}^{t} x(\tau)e^{\lambda(\tau-t)}d\tau$, so that

$$\frac{d}{dt}y(t) = x - \lambda y. \tag{8}$$

We now have the system

$$\begin{cases} \frac{dx}{dt} = f(t,x,y) \\ \frac{dy}{dt} = x - \lambda y \end{cases}. \tag{9}$$

B   The Goodwin Oscillator

The Goodwin Oscillator is a model of how feedback inhibition in biological systems can lead to oscillatory behavior, such as the behavior observed in circadian cycles [23]. In the Goodwin Oscillator model, each chemical species upregulates the next chemical species in a cycle except for the final chemical species, which downregulates the first species in a feedback step (Figure 1). The feedback term of the Goodwin Oscillator is a so-called Hill Function, which describes the kind of ligand-binding inhibitory events common to biochemical systems [24]. The general Goodwin Oscillator with n chemical species is said to be *of order n* and is given as follows:

$$\begin{cases} \frac{dx_1}{dt} = \frac{k_1}{1+x_n^\rho} - \phi_1 x_1 \\ \frac{dx_i}{dt} = k_i x_{i-1} - \phi_i x_i, \quad i \in \{2,\dots,n\}. \end{cases} \tag{10}$$

The power ρ indicates the degree of biochemical cooperativity in the ligand-binding reaction. Past work demonstrates that the Goodwin Oscillator always has a globally stable equilibrium state when $\rho = 1$, and that the there is a Hopf bifurcation to a stable limit cycle when $\rho \geq 2$ for sufficiently large n [23, 25].

The Goodwin Oscillator can be adjusted to allow for delay in the feedback step as follows:

$$\begin{cases} \frac{dx_1}{dt} = \frac{k_1}{1+z_n^\rho} - \phi_1 x_1 \\ \frac{dx_i}{dt} = k_i x_{i-1} - \phi_i x_i, \quad i \in \{2,\dots,n\}, \end{cases} \tag{11}$$
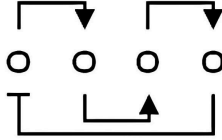
Figure 1: Schematic diagram of the Goodwin Oscillator. The negative feedback step is described by a Hill function and the positive feedforward steps are linear with constant coefficients.

where

$$z = \int_{-\infty}^{t} x_n(\tau) G(t - \tau) d\tau. \tag{12}$$

In the case where the distribution G is given by

$$G_a^p(u) = \frac{a^{p+1} u^p}{p!} e^{-au}, \tag{13}$$

it has been shown that the IDE system generated by this distributed DDE model is reducible to a system of ODEs [18]. Furthermore, the reduced ODE model is equivalent to a higher-order Goodwin Oscillator, so previous work on the stability of Goodwin Oscillators still applies [18, 23, 25].

C The Repressilator

A repressilator is a gene regulatory network containing an odd number of genes which forms a feedback loop wherein each gene expresses a protein which represses the following gene in the loop (Figures 2,4) [26]. Repressilators exhibit oscillatory behavior as each gene ultimately down regulates itself, with some time delay. The construction of the first synthetic repressilator heralded the creation of the field of *synthetic biology* [2]. The 3-repressilator contains three genes and may be represented with ODEs as follows:

$$\begin{cases} \frac{dx_i}{dt} = \frac{\alpha_i}{1+y_{i-1}^c} - \phi_i^{(1)} x_i \\ \frac{dy_i}{dt} = \beta_i m_i - \phi_i^{(2)} y_i, \quad i \in \{1,2,3\}, \end{cases} \tag{14}$$

where each x_i denotes an mRNA molecule and each y_i denotes a protein molecule [2]. If transcription and translation are binned, the same system may be approximately written as

$$\begin{cases} \frac{dy_1}{dt} = \frac{\alpha_1}{1+y_3^c} - \phi_1 y_1 \\ \frac{dy_2}{dt} = \frac{\alpha_2}{1+y_1^c} - \phi_2 y_2 \\ \frac{dy_3}{dt} = \frac{\alpha_3}{1+y_2^c} - \phi_3 y_3 \end{cases}. \tag{15}$$

In general, the (2n+1)-repressilator may be written as

$$\begin{cases} \frac{dy_1}{dt} = \frac{\alpha_1}{1+y_{2n+1}^c} - \phi_1 y_1 \\ \frac{dy_i}{dt} = \frac{\alpha_i}{1+y_{i-1}^c} - \phi_i y_i, \quad i \in \{2, \ldots, 2n+1\}. \end{cases} \tag{16}$$

As was the case with the Goodwin Oscillator, repressilators may be represented using distributed DDEs by introducing delays to each of the off-diagonal terms:

$$\begin{cases} \frac{dy_1}{dt} = \frac{\alpha_1}{1+z_{2n+1}^c} - \phi_1 y_1 \\ \frac{dy_i}{dt} = \frac{\alpha_i}{1+z_{i-1}^c} - \phi_i y_i, \quad i \in \{2, \ldots, 2n+1\}, \end{cases} \tag{17}$$

where

$$z_i = \int_{-\infty}^{t} y_i(\tau) G(t - \tau) d\tau. \tag{18}$$

Past work applying DDEs to the repressilator and related genetic circuit motifs has focused on discrete DDEs, however [27–29].

<div align="center">RESULTS</div>

A   The 1-Repressilator

The 1-repressilator consists of a single gene repressing its own expression (Figure 2). We may represent this system by binning transciption and translation and introducing distributed delays in the off-diagonal terms to derive the following *distributed delay differential equation* (distributed DDE):

$$\frac{dx}{dt} = \frac{k}{1 + z^{\rho}} - \phi x, \tag{19}$$

where

$$z = \int_{-\infty}^{t} x(\tau) G(t - \tau) d\tau \tag{20}$$

and G is a the distribution of the time delay. In the 1-repressilator case, this model is equivalent to the distributed DDE model of the Goodwin Oscillator of order one [18, 23].

We will consider the model in the special case where the delay distribution is given by

$$G_a^p(u) = \frac{a^{p+1} u^p}{p!} e^{-au}. \tag{21}$$

Let us denote

$$z_p = \int_{-\infty}^{t} x(\tau) G_a^p(t - \tau) d\tau, \tag{22}$$

so that

$$\frac{dx}{dt} = \frac{k}{1 + z_p^{\rho}} - \phi x. \tag{23}$$

Note that, in this case,

$$\frac{dG_a^p(u)}{du} = a(G_a^{p-1}(u) - G_a^p(u)). \tag{24}$$

Due to this fact and because $G_a^p(0) = 0$ for $p > 0$ and $G_a^p(0) = a$ for $p = 0$, we may write

$$\begin{cases} \frac{dz_p}{dt} = a(z_{p-1} - z_p), & p > 0 \\ \frac{dz_0}{dt} = a(x - z_0). \end{cases} \tag{25}$$

The entire system may therefore be reduced to the following system of *ordinary differential equations* (ODEs):

$$\begin{cases} \frac{dx}{dt} = \frac{k}{1+z_p^{\rho}} - \phi x \\ \frac{dz_0}{dt} = a(x - z_0) \\ \frac{dz_1}{dt} = a(z_0 - z_1) \\ \quad\vdots \\ \frac{dz_p}{dt} = a(z_{p-1} - z_p). \end{cases} \tag{26}$$
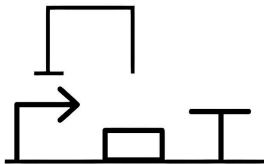
Pro   Gen   Ter

Figure 2: Schematic diagram of the 1-repressilator.

This is an ODE model of the Goodwin Oscillator of order $p + 2$ (Figure 1), so existing results regarding the stability of the Goodwin Oscillator apply. Namely, that the system will have stable limit cycles when $\rho \geq 2$ and $p + 2$ is sufficiently large [18].

The reduced ODE model may be numerically integrated using any numerical ODE solver. Numerical simulation reveals that the system does oscillate, as expected (Figure 3).

B   The 3-Repressilator

The 3-repressilator consists of three genes each repressing one another in a cycle (Figure 4). As in the 1-repressilator case, this system may be modeled as distributed DDE as follows:

$$\begin{cases} \frac{dx_1}{dt} = \frac{k_1}{1+z_{3,p}^\rho} - \phi_1 x_1 \\ \frac{dx_2}{dt} = \frac{k_2}{1+z_{1,p}^\rho} - \phi_2 x_2 \\ \frac{dx_3}{dt} = \frac{k_3}{1+z_{2,p}^\rho} - \phi_3 x_3, \end{cases} \tag{27}$$

where

$$z_p = \int\limits_{-\infty}^{t} x(\tau) G_a^p(t-\tau) d\tau \tag{28}$$

and

$$G_a^p(u) = \frac{a^{p+1} u^p}{p!} e^{-au}. \tag{29}$$

Analogously to the 1-repressilator case, we find that

$$\begin{cases} \frac{dz_{m,p}}{dt} = a(z_{m,p-1} - z_{m,p}) \\ \frac{dz_{m,0}}{dt} = a(x_m - z_{m,0}), \end{cases} \tag{30}$$
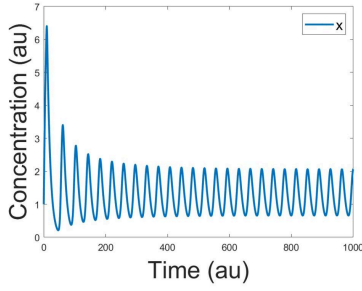
Figure 3: Numerical simulation of the reduced ODE representation of the 1-repressilator distributed DDE model. Oscillatory behavior does arise, as expected. $\phi = a = 0.1$, $k = 1$, $p = 4$, $\rho = 10$.
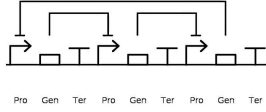
Figure 4: Schematic diagram of the 3-repressilator.

for $m \in \{1, 2, 3\}$. The distributed DDE model of the 3-repressilator therefore reduces to the following ODE model:

$$
\begin{cases}
\frac{dx_1}{dt} = \frac{k_1}{1 + z_{1,p}^\rho} - \phi_1 x_1 \\
\frac{dz_{1,0}}{dt} = a(x_1 - z_{1,0}) \\
\quad \vdots \\
\frac{dz_{1,0}}{dt} = a(z_{1,p-1} - z_{1,p}) \\
\frac{dx_2}{dt} = \frac{k_2}{1 + z_{1,p}^\rho} - \phi_2 x_2 \\
\frac{dz_{2,0}}{dt} = a(x_2 - z_{2,0}) \\
\quad \vdots \\
\frac{dz_{2,0}}{dt} = a(z_{2,p-1} - z_{2,p}) \\
\frac{dx_3}{dt} = \frac{k_3}{1 + z_{2,p}^\rho} - \phi_3 x_3 \\
\frac{dz_{3,0}}{dt} = a(x_3 - z_{3,0}) \\
\quad \vdots \\
\frac{dz_{3,0}}{dt} = a(x_{3,p-1} - z_{3,p}).
\end{cases}
\tag{31}
$$

Because this is a system of $3(p+2)$ ODEs, we will denote the 3-repressilator with no time delay as the $p=-1$ case, despite z_{-1} being poorly defined. Unfortunately, in contrast to the 1-repressilator case, the 3-repressilator is distinct from the Goodwin Oscillator when reduced, so existing stability results cannot be used.

In the steady state, this system satisfies

$$
\begin{aligned}
x_1 &= z_{1,0} = \cdots = z_{1,p} \\
x_2 &= z_{2,0} = \cdots = z_{2,p} \\
x_3 &= z_{3,0} = \cdots = z_{3,p}.
\end{aligned}
\tag{32}
$$

If we further impose that $k_1 = k_2 = k_3 = k$ and $\phi_1 = \phi_2 = \phi_3 = \phi$, then we can also conclude that

$$
x_1 = x_2 = x_3 = x.
\tag{33}
$$

At steady state, the systems must then satisfy

$$
x(1 + x^\rho) = \frac{k}{\phi}.
\tag{34}
$$

Because $x(1 + x^\rho)$ is a polynomial with a positive leading term, it always satisfies

$$
\lim_{x \to \infty} x(1 + x^\rho) \to \infty.
\tag{35}
$$

Furthermore, $x(1 + x^\rho)$ always has a root at 0 and is monotonically increasing for $x > 0$ because

$$
\frac{d}{dx}[x(1 + x^\rho)] = (\rho + 1)x^\rho + 1 > 0, \quad \text{for } x > 0.
\tag{36}
$$

Because $0 < \frac{k}{\phi} < \infty$ and $x(x^\rho + 1)$ has a root at zero and is monotonically increasing without bound for positive x, there must exist a unique positive solution to Equation 34. This value may be found computationally for given values of k, ϕ, and ρ (Table 1).

Under our assumption, with $p = 1$, the system Jacobian is given by

$$
J(x) =
\begin{pmatrix}
-\phi & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -\frac{k\rho x^{\rho-1}}{(1+x^\rho)^2} \\
a & -a & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & a & -a & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -\frac{k\rho x^{\rho-1}}{(1+x^\rho)^2} & -\phi & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & a & -a & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & a & -a & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -\frac{k\rho x^{\rho-1}}{(1+x^\rho)^2} & -\phi & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & a & -a & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & a & -a
\end{pmatrix},
\tag{37}
$$

and it is easy to extend the Jacobian for $p > 1$ by increasing the number of rows containing only a and $-a$ between the rows containing Hill functions. It is difficult to determine the eigenvalues of such matrices, in general, though the matrix may be amenable to specialized methods of analysis because it is almost-lower bidiagonal. As with the steady state, the

eigenvalues may be determined computationally given values for a, ϕ, k, ρ, and p (Table 1).

Table 1: Steady-state solution x and Jacobian eigenvalues for the 3-repressilator with simplifying assumptions ($k_1 = k_2 = k_3 = k$, $\phi_1 = \phi_2 = \phi_3 = \phi$). Positive, real steady-state solutions are unique. Equilibrium is marked as unstable if it has any eigenvalues with positive real part. In all cases, $\phi = a = 0.1$ and $k = 1$.

Parameter Values	Steady State x	Eigenvalues	Stability
$\rho = 1, p = -1$	2.7016	-0.1730 $-0.0635 + 0.0632i$ $-0.0635 - 0.0632i$	Stable
$\rho = 2, p = -1$	2	-0.2600 $-0.0200 + 0.1386i$ $-0.0200 - 0.1386i$	Stable
$\rho = 3, p = -1$	1.6975	-0.3491 $0.0245 + 0.2157i$ $0.0245 - 0.2157i$	Unstable
$\rho = 1, p = 0$	2.7016	$-0.1740 + 0.0427i$ $-0.1740 - 0.0427i$ $-0.1000 + 0.0854i$ $-0.1000 - 0.0854i$ $-0.0260 + 0.0427i$ $-0.0260 - 0.0427i$	Stable
$\rho = 2, p = 0$	2	$-0.2095 + 0.0632i$ $-0.2095 - 0.0632i$ $-0.1000 + 0.1265i$ $-0.1000 - 0.1265i$ $0.0095 + 0.0632i$ $0.0095 - 0.0632i$	Unstable
$\rho = 3, p = 0$	1.6975	$-0.2367 + 0.0789i$ $-0.2367 - 0.0789i$ $-0.1000 + 0.1578i$ $-0.1000 - 0.1578i$ $0.0367 + 0.0789i$ $0.0367 - 0.0789i$	Unstable
$\rho = 1, p = 1$	2.7016	-0.1900 $-0.1690 + 0.0579i$ $-0.1690 - 0.0579i$ $-0.1156 + 0.0887i$ $-0.1156 - 0.0887i$ $-0.0550 + 0.0780i$ $-0.0550 - 0.0780i$ $-0.0154 + 0.0308i$ $-0.0154 - 0.0308i$	Stable

		-0.2170	
		$-0.1896 + 0.0752i$	
		$-0.1896 - 0.0752i$	
		$-0.1203 + 0.1152i$	
$\rho = 2, p = 1$	2	$-0.1203 - 0.1152i$	Unstable
		$-0.0415 + 0.1013i$	
		$-0.0415 - 0.1013i$	
		$0.0099 + 0.0400i$	
		$0.0099 - 0.0400i$	
		-0.2356	
		$-0.2038 + 0.0871i$	
		$-0.2038 - 0.0871i$	
		$-0.1235 + 0.1335i$	
$\rho = 3, p = 1$	1.6975	$-0.1235 - 0.1335i$	Unstable
		$-0.0322 + 0.1174i$	
		$-0.0322 - 0.1174i$	
		$0.0274 + 0.0464i$	
		$0.0274 - 0.0464i$	
		$-0.1893 + 0.0239i$	
		$-0.1893 - 0.0239i$	
		$-0.1654 + 0.0654i$	
		$-0.1654 - 0.0654i$	
		$-0.1239 + 0.0893i$	
		$-0.1239 - 0.0893i$	
$\rho = 1, p = 2$	2.7016	$-0.0761 + 0.0893i$	Stable
		$-0.0761 - 0.0893i$	
		$-0.0346 + 0.0654i$	
		$-0.0346 - 0.0654i$	
		$-0.0107 + 0.0239i$	
		$-0.0107 - 0.0239i$	
		$-0.2086 + 0.0291i$	
		$-0.2086 - 0.0291i$	
		$-0.1795 + 0.0795i$	
		$-0.1795 - 0.0795i$	
		$-0.1291 + 0.1086i$	
		$-0.1291 - 0.1086i$	
$\rho = 2, p = 2$	2	$-0.0709 + 0.1086i$	Unstable
		$-0.0709 - 0.1086i$	
		$-0.0205 + 0.0795i$	
		$-0.0205 - 0.0795i$	
		$0.0086 + 0.0291i$	
		$0.0086 - 0.0291i$	

		$-0.2213 + 0.0325i$	
		$-0.2213 - 0.0325i$	
		$-0.1888 + 0.0888i$	
		$-0.1888 - 0.0888i$	
		$-0.1325 + 0.1213i$	
$\rho = 3, p = 2$	1.6975	$-0.1325 - 0.1213i$	Unstable
		$-0.0675 + 0.1213i$	
		$-0.0675 - 0.1213i$	
		$-0.0112 + 0.0888i$	
		$-0.0112 - 0.0888i$	
		$0.0213 + 0.0325i$	
		$0.0213 - 0.0325i$	

Computationally determining the steady state and its stability (Table 1) reveals that, like the Goodwin Oscillator, the 3-repressilator may bifurcate from stable to unstable if the degree of cooperativity ρ and system order p are increased. The numerical results suggest that, like the Goodwin Oscillator, the 3-repressilator may require $\rho \geq 2$ to become unstable. Simulation of the reduced ODE system corroborates this conjecture and also suggests that, like the Goodwin Oscillator, stable limit cycles appear when the fixed point becomes unstable (Figure 5).

C   Numerical Investigation

Simulation show that x_1, x_2, and x_3 tend to oscillate in-phase with one another as amplitude decays before reaching a stable fixed point or entering a stable limit cycle (Figure 5B,D). Further simulation reveals that initial conditions satisfying

$$[x_1(0), x_2(0), x_3(0)] \in \text{span}\{[1, 1, 1]\}$$

rapidly decay to the unique steady state, while other initial conditions sufficiently close to span$\{[1, 1, 1]\}$ oscillate in-phase while decaying in the $[1, 1, 1]$ direction before entering the stable limit cycle (Figure 6).

Visualization of several system trajectories in phase space shows that trajectories sufficiently close to span$\{[1, 1, 1]\}$ do indeed tend to oscillate in-phase and decay toward the steady state before falling onto the basin of attraction of the stable limit cycle. By contrast, initial conditions far outside the basin of attraction rapidly decay toward the stable limit cycle (Figure 7).
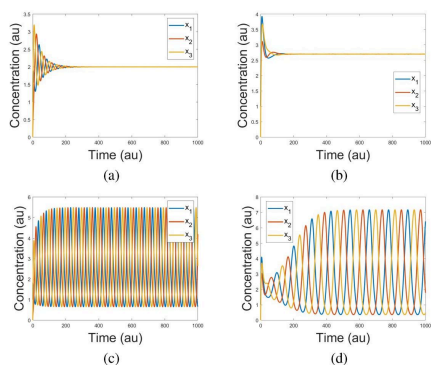
Figure 5: Simulation results for reduced ODE model of the 3-repressilator distributed DDE system. In all cases, $k = 1$ and $\phi = a = 0.1$. Simulation suggests that a stable limit cycle forms when the fixed point becomes unstable, as in the Goodwin Oscillator. Likewise, the simualtions show that increasing p or ρ may destabilize the fixed point, as in the Goodwin Oscillator. (a) Simulation results when $\rho = 2$ and $p = -1$, (b) $\rho = 1$ and $p = 0$, (c) $\rho = 3$ and $p = -1$, (d) and $\rho = 2$ and $p = 0$.

DISCUSSION

Genetic oscillators are the time-keeping devices of living organisms and are important in the study of circadian rhythms, the cell cycle, and developmental biology [3–5]. Despite their significance, modeling genetic oscillators in appreciable biological detail remains a challenge [11, 12]. Mathematical models of genetic oscillators tend to simplify the underlying biology, often neglecting important biological details in favor of simplicity and efficiency [13, 14]. Distributed delay differential equations are a promising approach to modeling genetic oscillators with greater biological detail [15]. To investigate the utility of distributed delay differential equations for modeling genetic oscillators, we constructed *distributed delay differential equation* (distributed DDE) models of genetic oscillatory motifs.

We found that distributed DDE models of the 1-repressilator (Figure 2) may be reduced to a system of *ordinary differential equations* (ODEs) given the delay distribution is of the
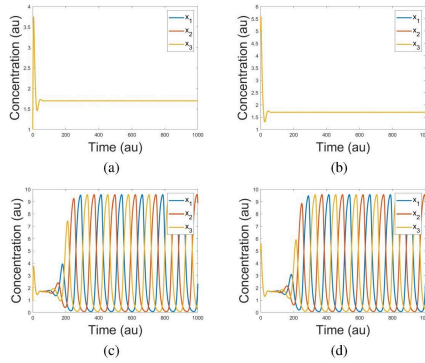
Figure 6: Simulation results for reduced ODE model of the 3-repressilator distributed DDE system. In all cases, $k = 1$, $\phi = a = 0.1$, $p = 0$, and $\rho = 3$. (a) Results for $[x_1(0), x_2(0), x_3(0)] = [1, 1, 1]$, (b) $[x_1(0), x_2(0), x_3(0)] = [5, 5, 5]$, (c) $[x_1(0), x_2(0), x_3(0)] = [1, 1, 0.99]$, (d) and $[x_1(0), x_2(0), x_3(0)] = [5, 5, 4.99]$

form

$$G_a^p(u) = \frac{a^{p+1}u^p}{p!}e^{-au}. \tag{38}$$

We showed that this reduced ODE system is equivalent to the previously-studied Goodwin Oscillator [23], for which stability criteria have previously been characterized [18]. In particular, the distributed DDE model reduces to the ODE model of a Goodwin Oscillator of higher order by introducing additional linear steps in the feedback circuit. These linear steps may be interpreted as representations of steps in gene expression which are often neglected in models, such as polyadenylation, pre-mRNA processing, and nuclear transport [30], relating these models to biological reality. Notably, the distributed delay system allows for oscillatory behavior given sufficiently large p (Figure 2), a feature of *in vivo* 1-repressilators which is not captured by ODE models [18, 23].

We also found that the 3-repressilator (Figure 4) may be reduced to a system of ODEs given the delay distribution is of the form of Equation 38. Again, this reduction introduced several feedforward steps between the feedback steps, potentially corresponding to increased biological detail. However, the 3-repressilator reduces to a system with more nonlinear terms than the Goodwin Oscillator and which is less understood. We showed
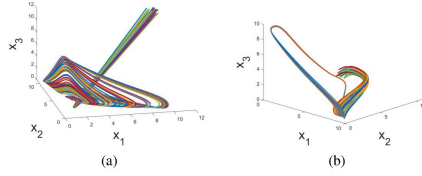
Figure 7: Phase space attractor diagrams of the 3-Repressilator distributed DDE system. In all cases, $k = 1$, $\phi = a = 0.1$, $p = 0$, and $\rho = 3$. (a) 50 trajectories are shown with initial conditions uniformly randomly generated in $[10, 11] \times [10, 11] \times [10, 11]$. Trajectories oscillate in phase near $\text{span}\{[1, 1, 1]\}$ before falling onto the basin of attraction. (b) 50 trajectories are shown with initial conditions uniformly randomly generated in $[0, 1] \times [10, 11] \times [0, 1]$. Trajectories rapidly fall onto the basin of attraction.

that, under some mild conditions, our model of the 3-repressilator has a unique positive, real equilibrium point. By linearizing the reduced ODE model about that unique positive, real equilibrium point for key parameter values, we showed that, under some conditions, increasing the degree of cooperativity ρ or the order of the reduced system p induced a bifurcation in which the equilibrium changes from stable to unstable (Table 1). In all cases, linearization (Equation 37) about the equilibrium point yielded conjugate-pair eigenvalues, indicating that oscillatory behavior exists in the neighborhood of the equilibrium point in both the stable and unstable case (Table 1). Numerical simulation results suggest that stable limit cycles exist in the large, only for parameter combinations with an unstable equilibrium point, as in the Goodwin Oscillator case (Figure 5).

Further numerical simulation revealed that the 3-repressilator exhibits a transient stable oscillatory response to initial conditions sufficiently close to $\text{span}\{[1, 1, 1]\}$ (Figure 7). If those initial conditions are within $\text{span}\{[1, 1, 1]\}$, then they rapidly converge to the constant steady-state solution. Otherwise, they decay toward the steady-state solution before falling onto the basin of attraction of a stable limit cycle, given that parameters are selected such that one exists (Figure 6). By contrast, initial conditions sufficiently far from $\text{span}\{[1, 1, 1]\}$ rapidly decay toward the stable limit cycle without oscillating around the stable limit cycle (Figure 7).

There is room for much future work in this area. By assuming a delay distribution of the form of Equation 38, we were able to reduce both the 1-repressilator and the 3-repressilator to ODE models, however, we lack a biophysical motivation for why the delay distribution ought to be of a particular form. Further investigation could yield a more motivated distribution to study. Additionally, we argue that the feedforward steps introduced to the ODE models of both repressilators by the distributed delays may correspond to more granular biological detail, however, these feedforward steps all share a coefficient a. Our models

must be validated on experimental data to determine whether this limitation reduces their ability to model biological phenomena well.

We were able to propose some conditions for stability and oscillatory behavior in the 3-repressilator case based on the results of numerical simulation (Table 1, Figure 5), but we did not provide proof of these proposals. Further study of the behavior of our 3-repressilator model is therefore necessary. A more analytical approach could perhaps also be generalized to the case of the (2n+1)-repressilator.

Our study of distributed DDEs for the modeling of genetic oscillators revealed several key properties of such models as well as a method of deriving equivalent reduced ODE models. These reduced ODE models allow for simulation of the delay systems using traditional numerical integration techniques, enabling further study of such systems as well as their applications to genetic design tasks. With detailed biological models of this kind, emergent phenomena related oscillatory biological behaviors may be probed, and novel synthetic biological devices may be designed.

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REFERENCES

[1]   Deborah Bell-Pedersen, Vincent M. Cassone, David J. Earnest, Susan S. Golden, Paul E. Hardin, Terry L. Thomas, and

Mark J. Zoran. Circadian rhythms from multiple oscillators: Lessons from diverse organisms. *Nature Reviews Genetics*, 6(7):544–556, 2005.

[2]   Michael B. Elowitz and Stanislas Leibler. A synthetic oscillatory network of tran- scriptional regulators. *Nature*, 403(6767):335–338, 2000.

[3]   P Smolen and JH Byrne. Circadian rhythm models. *Encyclopedia of Neuroscience*, pages 957–963, 2009.

[4]   Sean Crosson, Harley McAdams, and Lucy Shapiro. A genetic oscillator and the regulation of cell cycle progression in caulobacter crescentus. *Cell Cycle*, 3(10):1252– 1254, 2004.

[5]   Charisios Tsiairis and Helge Großhans. Gene expression oscillations in c. elegans underlie a new developmental clock. In *Current Topics in Developmental Biology*, volume 144, pages 19–43. Elsevier, 2021.

[6]   Martha Hotz Vitaterna, Joseph S Takahashi, and Fred W Turek. Overview of circadian rhythms. *Alcohol research & health*, 25(2):85, 2001.

[7]   Oliver Purcell, Nigel J Savery, Claire S Grierson, and Mario Di Bernardo. A compar- ative analysis of synthetic genetic oscillators. *Journal of The Royal Society Interface*, 7(52):1503–1524, 2010.

[8]   Peng-Fei Xia, Hua Ling, Jee Loon Foo, and Matthew Wook Chang. Synthetic ge- netic circuits for programmable biological functionalities. *Biotechnology advances*, 37(6):107393, 2019.

[9]   Cong Gao, Peng Xu, Chao Ye, Xiulai Chen, and Liming Liu. Genetic circuit-assisted smart microbial engineering. *Trends in microbiology*, 27(12):1011–1024, 2019.

[10]   Pu Shi, Joshua A Gustafson, and J Andrew MacKay. Genetically engineered nanocar- riers for drug delivery. *International journal of nanomedicine*, 9:1617–1626, 2014.

[11]   Alexander N Churilov, Alexander Medvedev, and Zhanybai T Zhusubaliyev. Delay- induced dynamical phenomena in impulsive goodwin's oscillator: What we know so far. In *2015 54th IEEE Conference on Decision and Control (CDC)*, pages 590–595. IEEE, 2015.

[12]   Michael A Gibson and Jehoshua Bruck. Efficient exact stochastic simulation of chem- ical systems with many species and many channels. *The journal of physical chemistry A*, 104(9):1876–1889, 2000.

[13]   Payton J Thomas, Mohammad Ahmadi, Hao Zheng, and Chris J Myers. A comparison of weighted stochastic simulation methods. *bioRxiv*, pages 2021–09, 2021.

[14]   Mohammad Ahmadi, Payton J Thomas, Lukas Buecherl, Chris Winstead, Chris J Myers, and Hao Zheng. A comparison of weighted stochastic simulation methods for the analysis of genetic circuits. *ACS Synthetic Biology*, 2022.

[15]   Marcella Gomez and Richard M Murray. Delay-based approximations of biological systems for analysis and design. In *Am. Contr. Conf.*, 2012.

[16]   Franco Blanchini and Elisa Franco. Structural analysis of biological networks. *A Systems Theoretic Approach to Systems and Synthetic Biology I: Models and System Characterizations*, pages 47–71, 2014.

[17]   David S Glass, Xiaofan Jin, and Ingmar H Riedel-Kruse. Nonlinear delay differen- tial equations and their application to modeling biological network motifs. *Nature communications*, 12(1):1788, 2021.

[18]   N MacDonald. Time lag in a model of a biochemical reaction sequence with end product inhibition. *Journal of Theoretical Biology*, 67(3):549–556, 1977.

[19]   Marc R Roussel. Delay-differential equations. In

Nonlinear Dynamics, 2053-2571, pages 12–1 to 12–14. Morgan Claypool Publishers, 2019.

[20]   Jean-Pierre Richard. Time-delay systems: an overview of some recent advances and open problems. *Automatica*, 39(10):1667–1694, 2003.

[21]   Philip Doldo and Jamol Pender. A note on the interpretation of distributed delay equations, 2021.

[22]   Luo Albert C J. and Jian-Qiao Sun. *Chapter 3: Differential-Delay Equations*, page 85–119. Springer Berlin Heidelberg, 2012.

[23]   Brian C. Goodwin. Oscillatory behavior in enzymatic control processes. *Advances in Enzyme Regulation*, 3:425–437, 1965.

[24]   Rudolf Gesztelyi, Judit Zsuga, Adam Kemeny-Beke, Balazs Varga, Bela Juhasz, and Arpad Tosaki. The hill equation and the origin of quantitative pharmacology. *Archive for History of Exact Sciences*, 66(4):427–438, 2012.

[25]   Maya Mincheva and Marc R. Roussel. Turing-hopf instability in biochemical reaction networks arising from pairs of subnetworks. *Mathematical Biosciences*, 240(1):1–11, 2012.

- [26] Samuel M. D. Oliveira, Jerome G. Chandraseelan, Antti Ha¨kkinen, Nadia S. M. Goncalves, Olli Yli-Harja, Sofia Startceva, and Andre S. Ribeiro. Single-cell ki- netics of a repressilator when implemented in a single-copy plasmid. *BioSyst.*, 11:1939–1945, 2015.

- [27] Mehmet Eren Ahsen, Hitay O¨ zbay, and Silviu-Iulian *Deterministic ODE- Based Model with Time Delay*, pages 43–51. Springer International Publishing, Cham, 2015.

- [28] Parmar, K. B. Blyuss, Y. N. Kyrychko, and S. J.

Hogan. Time-delayed models of gene regulatory networks. *Computational and Mathematical Methods in Medicine*, 2015:1–16, 2015.

- [29] Chen and K. Aihara. Stability of genetic regulatory networks with time delay. *IEEE Transactions on Circuits and Systems I: Fundamental Theory and Applications*, 49(5):602–608, 2002.

- [30] Murray Polyadenylation and nuclear export of mrnas. *Journal of Biological Chemistry*, 294(9):2977–2987, 2019.

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111. Exploring Novel PI3K-AKT-MTOR Therapy Inhibition as a Treatment for Braf-Mutant Melanoma

Ashley Thompson; Gennie L. Parkman (School of Biological Sciences); and Sheri L. Holmen (Hunstman Cancer Institute)

Faculty Mentor: Gennie L. Parkman (Biological Sciences, University of Utah)

Even with the emergence of new melanoma therapies, melanoma progression and metastasis is still the leading cause of death for those suffering from skin cancer (Liu, et al. 2014). There is only a 15-20% five-year survival rate for Stage IV melanoma, thus proving the need for new target therapies for melanoma. The phosphatidylinositol-3'-kinases (PI3K) and mitogen-activated protein kinase (MAPK) pathways play a significant role in cell proliferation in normal and cancer cells. PI3K and MAPK phosphorylate downstream effectors that regulate many biochemical processes during cell proliferation. In the MAPK pathway, a protein kinase called BRAF is the most commonly mutated proto-oncogene found in melanoma metastasis. However, mutation of this gene alone does not lead to melanomagenesis (Parkman, et al. 2021). Instead, melanomagenesis requires other mutations in various cell proliferation pathways such as the PI3K pathway. Some of the downstream effectors of the PI3K pathway are AKT and serum-and-glucocorticoid-regulated kinase (SGK). AKT has many roles in cell proliferation but most notably promotes activation of mTORC1, a protein that onsets cell proliferation. SGK is highly homologous to AKT. It has many of the same downstream effectors and promotes the activation of mTORC1 (Sommer, et al. 2013). Many current melanoma therapies target the MAPK pathway and particularly the commonly mutated BRAF protein. These therapies have shown success in inhibiting the MAPK pathway; nevertheless, the PI3K pathway is resistant to these therapies and remains active and able to continue cell growth. To combat

the activation of the PI3K pathway, therapeutics have been developed to inhibit AKT and PI3K. However, the PI3K and AKT-targeted therapeutics are not successful in reducing the progression of melanoma metastasis and thus have not been FDA-approved. When AKT is genetically inhibited, overexpression of SGK occurs and can rescue the knockdown of AKT (unpublished data from the Holmen lab). Therefore, to decrease the proliferation effect of the PI3K pathway, the combination of inhibition of AKT and SGK is warranted. We report pharmacological inhibitors against both AKT and SGK to decrease melanoma cell proliferation in vitro. Furthermore, the combination of AKT + SGK inhibition resulted in decreased tumor progression and increased overall survival ($p=0.0031$) in a BRAFV600E-driven immunocompetent mouse model of melanoma. These findings demonstrate that dual targeting of SGK and AKT may represent a novel therapeutic strategy to diminish tumor growth.

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112. Research Reflection by Ashely Thompson

Ashley Thompson

Faculty Mentor: Gennie L. Parkman (Biological Sciences, University of Utah)

My future goals are to attend medical school and become a doctor. As of now, I am interested in becoming a dermatologist. My undergraduate research experience on melanoma has helped me brush the surface of the vast topic of skin cancer. Additionally, my experience has helped me form connections with practicing dermatologists. Those connections allowed me to shadow a dermatologist and see surgeries, diagnoses, and treatment plans. I was also able to build relationships with my lab members and mentors which helped me not only grow as a researcher but as a person.

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113. Optimization of Cobalt Catalysts in the Hydrogenation of Carbon Dioxide to Methanol

Rachel Whipple; Caroline T. Saouma (Chemistry); and Clara Wiesler

Faculty Mentor: Caroline Saouma (Chemistry, University of Utah)

As greenhouse gas emissions increase, the capture of carbon dioxide has become a point of interest. This captured CO_2 can be utilized as a cheap, abundant carbon source that can then be converted back to fuels, such as methanol, which is currently produced on a large-scale globally [1]. Current methods to

produce MeOH emit greenhouse gases, so CO2 conversion to MeOH would be beneficial as it would both reduce CO2 emissions and utilize the CO2 that is already in the atmosphere.

While this conversion has been proven possible, it has been accomplished with heterogeneous catalysts that require elevated temperature and pressure to produce methanol. Homogenous catalysts can react under milder conditions, but are often made from non-abundant metals, such as ruthenium [2,3]. Exploring the possibility of using more earth-abundant metals, such as cobalt, to form the catalyst has been done, but these have been ill-defined catalysts [1]. To optimize these cobalt catalysts and investigate the catalytic cycle, we are synthesizing a variety of well-defined homogeneous complexes and proposed cobalt(I) and cobalt(II) hydride intermediates based on a cobalt-triphos system. The catalytic performance of methanol production of these catalysts is then compared to each other, and the ill-defined catalysts used previously to determine the most effective catalysts. Preliminary results indicate MeOH is made, and ongoing work will more accurately quantify the results.

[1] Schneidewind, J.; Adam, R.; Baumann, W.; Jackstell, R.; Beller, M. Low-Temperature Hydrogenation of Carbon Dioxide to Methanol with a Homogeneous Cobalt Catalyst. Angew. Chem. Int. Ed Engl. 2017, 56 (7), 1890-1893.

[2] Kothandaraman, J.; Goeppert, A.; Czaun M.; Olah G. A.;Prakash G. K. S. Conversion of Co2 from Air into Methanol Using a Polyamine and a Homogenous Ruthenium Catalyst. JACS. 2016, 138 (3), 778-781.

[3] Wesselbaum, S.; Vom Stein, T.; Klankermayer, J.; Leitner, W. Hydrogenation of carbon dioxide to methanol by using a

homogeneous ruthenium-phosphine catalyst. Angew Chem Int Ed Engl. 2012, 51 (30), 7499-7502.

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114. A Catalog of Nearby Accelerating Star Candidates in Gaia DR3

Marc Whiting; Ben Bromley (Physics & Astronomy); Josh Hill; and Scott Kenyon (Harvard University)

Faculty Mentor: Ben Bromley (Physics & Astronomy, University of Utah)

We present a new compilation of accelerating star candidates, known as the Gaia Nearby Accelerating Star Catalog (GNASC), featuring 29,684 candidates within 100 pc and brighter than Gaia G 17.5 mag. This catalog was developed using a supervised machine learning algorithm, drawing data

from the Hipparcos-Gaia Catalog of Accelerations (HGCA), Gaia Data Release 2, and Gaia Early Data Release 3. Our method exploits the difference in observation periods between Gaia catalogs and astrometric modeling quality, assuming a correlation with acceleration.

Membership in the catalog is determined by confidently ruling out constant proper motion over 30 years (greater than 99.9% confidence). According to test data, each catalog member has a 68% chance of actual astrometric acceleration, with some subsets exceeding 85%. We compared GNASC to Gaia Data Release 3, capturing over 96% of sources that fit our criteria in its high-confidence acceleration table.

Our catalog includes bright, nearby candidates not present in the initial Hipparcos survey, comprising members of known binary systems and stars with yet-to-be-identified companions. GNASC extends the HGCA and highlights the potential of machine learning in uncovering hidden stellar partners in future astrometric surveys.

References

Astropy Collaboration, Price-Whelan, A. M., Sipőcz, B. M., et al. 2018, AJ, 156, 123 Belokurov, V., Penoyre, Z., Oh, S., et al. 2020, MNRAS, 496, 1922 Bessel, F. W. 1844, MNRAS, 6, 136 Bond, G. 1862, AN, 57, 131 Brandt, G. M., Brandt, T. D., Dupuy, T. J., Li, Y., & Michalik, D. 2021, AJ, 161, 179 Brandt, G. M., Dupuy, T. J., Li, Y., et al. 2021, AJ, 162, 301 Brandt, T. D. 2018, ApJS, 239, 31 Brandt, T. D. 2021, ApJS, 254, 42 Brandt, T. D., Dupuy, T. J., & Bowler, B. P. 2019, AJ, 158, 140 Cantat-Gaudin, T., & Brandt, T. D. 2021, A&A, 649, A124 Chulkov, D., & Malkov, O. 2022, MNRAS, 517, 2925 Currie, T., Brandt, T. D., Kuzuhara, M., et al. 2020, ApJL, 904, L25 De Rosa, R. J., Nielsen, E. L., Wahhaj, Z., et al. 2023, arXiv:2302.06332 Djurašević, G., Yılmaz, M., Baştürk, Ö., et al. 2011, A&A, 525, A66 ESA 1997, ESA

SP-1200, The Hipparcos Catalog (Noordwijk: ESA) Fuhrmann, K., Chini, R., Kaderhandt, L., & Chen, Z. 2017, ApJ, 836, 139 Gaia Collaboration, Brown, A. G. A., Vallenari, A., et al. 2018, A&A, 616, A1 Gaia Collaboration, Brown, A. G. A., Vallenari, A., et al. 2021, A&A, 649, A1 Gaia

Collaboration, Prusti, T., de Bruijne, J. H. J., et al. 2016, A&A, 595, A1 Gaia Collaboration, Vallenari, A., Brown, A. G. A., et al. 2022, arXiv:2208. 00211 Galle, J. G. 1846, MNRAS, 7, 153 Halbwachs, J.-L., Pourbaix, D., Arenou, F., et al. 2022, arXiv:2206.05726 Holl, B., Sozzetti, A., Sahlmann, J., et al. 2022, arXiv:2206.05439 Kervella, P., Arenou, F., & Thévenin, F. 2022, A&A, 657, A7 Kuzuhara, M., Currie, T., Takarada, T., et al. 2022, ApJL, 934, L18 Latković, O., Čeki, A., & Lazarević, S. 2021, ApJS, 254, 10 Lindegren, L. 2020, A&A, 633, A1 Lindegren, L., Klioner, S. A., Hernández, J., et al. 2021, A&A, 649, A2 Lohr, M. E., Norton, A. J., Payne, S. G., West, R. G., & Wheatley, P. J. 2015, A&A, 578, A136 Lubin, J., Robertson, P., Stefansson, G., et al. 2021, AJ, 162, 61 McCarthy, K., & White, R. J. 2012, AJ, 143, 134 Pearce, L. A., Males, J. R., Weinberger, A. J., et al. 2022, MNRAS, 515, 4487 Perryman, M. A. C., Lindegren, L., Kovalevsky, J., et al. 1997, A&A, 323, L49 Ribas, I., Tuomi, M., Reiners, A., et al. 2018, Natur, 563, 365 Riedel, A. R., Finch, C. T., Henry, T. J., et al. 2014, AJ, 147, 85 Stassun, K. G., & Torres, G. 2021, ApJL, 907, L33 Stępień, K. 2009, MNRAS, 397, 857 Struve, M. O. 1873, MNRAS, 33, 430 Sutlieff, B. J., Bohn, A. J., Birkby, J. L., et al. 2021, MNRAS, 506, 3224 Tokovinin, A. 2017, ApJ, 844, 103 Turon, C., Requieme, Y., Grenon, M., et al. 1995, A&A, 304, 82 van de Kamp, P. 1956, VA, 2, 1040 van de Kamp, P. 1969, AJ, 74, 238 Vrijmoet, E. H., Henry, T. J., Jao, W.-C., & Dieterich, S. B. 2020, AJ, 160, 215 Vrijmoet, E. H., Tokovinin, A., Henry, T. J., et al. 2022, AJ, 163, 178

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115. Research Reflection by Marc Whiting

Marc Whiting

Faculty Mentor: Ben Bromley (Physics & Astronomy, University of Utah)

Returning to my academic pursuits, I never expected to be immersed in such an extraordinary and singular research project. Working alongside my esteemed colleague Josh Hill and esteemed mentors Ben Bromley and Scott Kenyon has been pivotal to my intellectual growth and expansion. Little did I know that such incredible opportunities awaited diligent undergraduate students like Josh and I. We both feel profoundly grateful for the invaluable collaborative efforts that have positively impacted our community.

The education and hands-on experience we have all acquired working together throughout the past year has fueled significant personal and professional development. This research endeavor has deepened our understanding of the

subject matter and fortified my future ambitions. I eagerly anticipate the new directions this acquired knowledge and experience will bring.

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116. Environmental Analysis of Juvenile Versus Adult Ungulate Habitat Use in the Wasatch Mountain Range

MacKenzie Woodrum and Austin Green

Research Mentor: Austin Woodrum (University of Utah)

Human Influence is becoming increasingly ubiquitous across most landscapes in North America, forcing wildlife to adapt to ever-changing situations to persist. In this light, it is critical that scientists understand how wildlife behavior and distribution is affected by human influence. Recent research suggests that wildlife adapt to human influence in different ways, largely based on evolutionary taxonomy and life history

characteristics, highlighting how interspecific variation in human influence response leads to differences in wildlife community structure across a wild to urban gradient. However, there is little work done on how individual species' responses to human influence vary across major life stages (e.g., when raising young vs. when dispersing to new environments). Therefore, in this study, we will investigate how ungulates in the Wasatch Mountain range use their environment during different life stages. We will do this by comparing the distribution and habitat preferences of adults vs. juveniles with and without an accompanying adult. We hypothesize that species distributions and habitat preferences, including responses to human influence factors, will vary across life stages. We predict that the presence of juveniles will make species more likely to avoid areas of higher risk, like areas rich with human development and recreational activity. This research will highlight the importance of studying wildlife during different life stages, making it possible for wildlife managers to better understand what elements of an environment are critical to juvenile development.

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117. Research Reflection by MacKenzie Woodrum

MacKenzie Woodrum

My undergraduate research has given me the opportunity to gain unique experience in a field that interest me. By doing this research I hope to make an impact on conservations efforts in hopes to better protect our wildlife populations. Once we can determine the elements of the environment which are critical to population growth we can ensure the preservation of our wildlife. These conservation efforts have given me a greater desire to help all animals. I hope to go to veterinary school to keep helping a wide variety of our animals.

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118. In Darwin's Footsteps: A Shared Genetic Control for Toe and Beak Length in Colombia Livia

Bailey Young and Michael Shapiro (School of Biological Sciences(

Faculty Mentor: Michael D. Shapiro (School of Biological Sciences, University of Utah)

Domestic rock pigeons (Columba livia) display an incredible amount of variation among different breeds. Even though they can look and act differently, these breeds all belong to the same species. We are therefore able to breed individuals with very different traits and perform genetic mapping. In The Variation

of Animals and Plants Under Domestication, Darwin observed that the data he collected "indicate pretty plainly some kind of correlation between the length of the beak and the size of the feet" (Darwin, 1868). The goal of my research is to determine whether there is a shared genetic control of foot size and beak size in domestic rock pigeons. First, I collected limb length measurements from the F2 generation of a cross between a Homer (medium beaked) and an Old German Owl (small beaked) pigeon. This cross segregates different beak lengths and variation at a locus on Chromosome Z, ROR2, is linked to beak shortening (Boer et al., 2021); therefore, this cross presents an ideal opportunity to test for anatomical and genetic associations between beak and toe lengths. My anatomical data confirmed that foot and beak size are indeed associated. Next, I used quantitative trait locus (QTL) mapping and found that toe size is controlled by at least two genetic loci on linkage group Z and 8, one of which maps to the same genomic region that controls beak length. Therefore, it is likely that toe size and beak length are controlled by the same or closely linked genes. Thus, variation in one genomic region – and possibly one gene – can lead to coordinated changes in seemingly unrelated anatomical structures.

Bibliography

Boer, E. F., Van Hollebeke, H. F., Maclary, E. T., Holt, C., Yandell, M., & Shapiro, M. D. (2021). A ROR2 coding variant is associated with craniofacial variation in domestic pigeons. Current Biology, 31(22), 5069-5076.e5. https://doi.org/10.1016/j.cub.2021.08.068

Darwin, C. (1868). The Variation of Animals and Plants Under Domestication (Vol. 1). John Murray.

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SECTION XII

Social and Behavioral Science

119. **Educating and Engaging Youth about the Equal Rights Amendment Through Legislative Advocacy**

Tiffany Chan

Faculty Mentor: James Curry (Political Science, University of Utah)

Introduction

As a community organizer and a relentless advocate for social justice, I realized early on that gender issues would be deeply ingrained in my activism. As a woman of color, I was exposed to the ugly truths of gender gaps and forms of prejudice and

discrimination perpetuated by sexists, misogynists, and male chauvinists. It was already burdensome to bear the brunt of these behaviors, but it's more challenging when there's no legal guarantee within the Constitution for a fundamental legal remedy against sex discrimination by guaranteeing that constitutional rights may not be denied or abridged on account of sex.

When the U.S. Constitution was adopted in 1787, the rights it affirmed were guaranteed equally only for certain white males. After intense political battles and a bloody civil war, those rights have been extended far more broadly through constitutional amendments, laws, and court decisions for race and other protected classes. However, all of those rights are not yet guaranteed to apply equally without regard to sex. The first — and still the only — right that the U.S. Constitution specifically affirms and applies equally to women and men is the right to vote. The equal protection clause of the U.S. Constitution's 14th Amendment was first applied to sex discrimination only in 1971, and according to the Equal Rights Amendment website, it has never been interpreted to grant equal rights on the basis of sex in a uniform and inclusive way ("Why We Need the Equal Rights Amendment").

The Equal Rights Amendment (ERA) is designed to guarantee equal legal rights for all American citizens regardless of sex. It is necessary because the Constitution has never been interpreted to guarantee the rights of women as a class, and the

rights of men as a class, to be equal. It seeks to end the legal distinctions between men and women in terms of divorce, property, employment, and other matters. For the first time, sex would be considered a suspect classification, as race, religion, and national origin currently are. Governmental actions that treat males or females differently as a class would be subject to strict judicial scrutiny and would have to meet the highest level of justification – a necessary relation to a compelling state interest – to be upheld as constitutional ("Why We Need the Equal Rights Amendment"). The ERA would guarantee "Equal Justice Under Law," as inscribed over the entrance to the Supreme Court, and send a strong preemptive warning against writing, enforcing, or adjudicating laws unfairly on the basis of sex.

The ERA would provide a clearer judicial standard for deciding cases of sex discrimination. Not every state in the U.S. has ratified the Equal Rights Amendment, and therefore federal and state courts are inconsistent in their rulings regarding claims of sexual discrimination. The ERA would help clarify sex discrimination jurisprudence and would provide a strong legal defense against a rollback of the significant advances in women's rights that have been achieved since the mid–20th century. Without the ERA women regularly — and occasionally men — have to fight long, expensive, and difficult legal battles in an effort to prove that

their rights are equal to those of the other sex. Without the ERA, the U.S. Constitution does not explicitly guarantee that the rights it protects are held equally by all citizens without regard to sex.

The longer the ERA is not ratified in Utah, the longer that many live without equal rights indefinitely, which is why this is pertinent to the youth as they are changemakers and help change the future of the country. More social changes that have come with time have helped lean away from patriarchal culture as young Americans learn to incorporate more social justice into their timelines, such as the Me Too movement that revealed ugly truths and the urgent need for women's rights that sparked national conversations and protests. As solidarity becomes more championed, it gradually becomes an inherent characteristic through collective organizing, effectively building power and influence of youth activists. Thus, the youth are needed because they can help drive another shift towards an equitable future and could prove to be a powerful and collective force for leveling up the ERA ratification.

Literature Review
Introduction

The Equal Rights Amendment relies on the push for ratification that has been ongoing for decades. It had begun with strong support but was also met with organized opposition from conservatives that had stopped the ERA 3 short of the needed 38 state

ratifications (Cohen and Codrington, 2020). Since 1978, attempts have been made in Congress to extend or remove the deadline for ratification. In the 2010s, due in part to fourth-wave feminism and the Me Too movement, interest in getting the ERA adopted was revived. The main pillars to these renewed efforts are focused on education, advocacy, and activism that targets today's youth and decision-making legislators. Therefore, exploring scholarships about 1) the importance of youth engagement in legislative advocacy and the obstacles to it, and 2) what can motivate legislators to take action on what issues, will help understand why the effort to ratify the ERA is centered around these two pillars.

Engaging in Legislative Advocacy

Social movement scholars have long realized that young people have been, and continue to be, critical to the rise of many social movements and social movement organizations (SMOs) over the last 50 years (Earl, Maher, & Elliott, 2017, p. 2). Student contributions include (but are not limited to) the New Left, the Free Speech movement, lunch counter sit-ins to push for desegregation, campus campaigns for anti-apartheid divestment, anti-sweatshop activism, the DREAMers, and Black Lives Matter. It is hard to imagine the landscape of activism in the United States without the efforts of young people.

Nevertheless, engaging youth in legislative

advocacy is difficult. Barriers to youth political engagement are erected by assumptions that youth are not interested in politics, which denies young people agency over their own political socialization. Youth political participation is also undermined by the fact that young people have fewer of the resources that predict political engagement: money, time, knowledge, skills, and efficacy (Rosenstone & Hansen, 2010, p. 402). Moreover, those who have a lower socioeconomic status and less privileged backgrounds tend to participate less and are less engaged. This is especially true for female and BIPOC youth who, traditionally, have been disproportionately absent from the political organizing scene. They face more systemic issues that prevent them access to advocacy as well as dismissal of their political agency. Youth of color face these barriers to participation within activist organizations, too. Only a few organizations engage in intersectional mobilization and recruitment strategies (Elliott, Earl, & Maher, 2017, p. 6), leading to a lack of opportunities targeted explicitly to people of color. Youth of color are likely to experience a double penalty in this regard, both for their age and for their racial and ethnic identities. These barriers are especially consequential for youth of color, because of the social capital these youth gain from participating in community and activist organizations.

Youth activism has undergone recent transformation to its participants and forms of legislative advocacy, which could prove beneficial if the increased mobilizing capabilities are strategically used and recognized. The intersectional identities of many young people draws together political socialization, youth participation in activist organizations, and social movements more broadly by focusing on the gendered and racialized experiences of youth activists (Fisher, 2012, p. 124). Girls are often faced with a general activist identity that devalues their identities as girls, forcing them to do additional identity work to make their identities congruent and work to legitimize a girl identity within their activist identity. Further, the increasingly tumultuous political context around race has driven a new generation to become involved in social justice activism, drawing on intersectional approaches to youth and racial identities.

Motivating Factors for Legislators

Legislators estimate the costs and benefits of time spent with an advocacy organization or group. Potential benefits legislators seek from providing an advocacy organization access fall under three categories: electoral, informational, and intrinsic.

Electoral benefits relate to how much electoral payoff a legislator can expect by working with a particular issue group (Wiener, 2020, p. 3). A legislator is likely to estimate attractive benefits

from providing access to or partnering with a group with strong capacities to mobilize his or her constituents to vote or to contribute to his or her campaign (Mayhew, 1974; Arnold, 1990). Informational benefits are more broadly defined, as valuable information can take many forms (Wiener, 2020, p. 3). But groups can often provide valuable information to a legislator, including about the opinions of groups of voters, or about how stakeholders in the public are affected by existing policies. Finally, intrinsic benefits can be had from the positive or negative feelings a legislator gets from working with or on behalf of a particular group (Broockman, 2013, p. 522). These benefits relate to a legislator's personal preferences and life experiences. For state legislatures like Utah's that are predominantly white and male, their identities don't match with marginalized groups' interests. For a marginalized group facing structural barriers to access, intrinsic benefits become a critical factor to consider, as a legislator's own experience of marginalization as a member of an identity group can shape their political priorities.

Ample research sees this work out in practice. Legislators, especially those from historically marginalized groups, are found to prioritize the interests of their identity groups in solidarity (Gay, 2004, p. 547)–a phenomena called "descriptive representation." This effect is found among women legislators, BIPOC legislators, LGBTQ legislators,

and more. Research also finds that women act differently than their male colleagues in legislatures. Women bring gendered life experiences to political institutions, ultimately reflecting different interests than their male counterparts. In state legislatures specifically, Holman and Mahoney (2018) find that the presence of a woman's caucus leads to increases in women's collaboration on women's interest legislation (even across party lines).

Interestingly, it is not just the presence of White and minority women alone that makes political institutions more responsive to women's issues, but rather it is the organizational presence of minority men along with minority women whose ideals about women's issues are similar or align with each other. Much of the liberalization of minority men is due to the existence of the interaction with minority women in caucuses (Minta & Brown, 2014, p. 257). Organizations such as the Congressional Black Caucus (CBC), Congressional Hispanic Caucus (CHC), and Congressional Asian and Pacific Islander Caucus (CAPAC) were formed by minority men and women legislators to represent the interests of Blacks, Latinos, and Asian Americans. The result of this is that the presence of women in minority caucuses has an effect on the men in minority caucuses who become advocates for women's issues. Intersectionality of gender and racial diversity in congressional action have led to

more attention to issues that directly and indirectly impact women.

Conclusion

Studying these two areas will strengthen my knowledge on how to best effectively mobilize stronger advocacy and support for the Equal Rights Amendment today. A more actively engaged youth will draw more attention from legislators, which in turn could reshape the positions legislators hold on the issues, including the ERA. Given the large influence young activists have, this will help me understand how to push white and male legislator majority to weigh the benefits, such as electoral and informational, to work alongside ERA efforts. With Utah being one of the states that has not yet ratified the Equal Rights Amendment, these efforts are worthwhile and will prove to be a historic step towards equality in the conservative state.

Project Narrative

Key Partners

There are several key players that are critical for the success of this project. They include the Utah ERA Coalition and its members/affiliates. I reached out to this organization via email after I saw an event organized on Utah Capitol Hill and was connected with my future Community Partner (CP), Kelly Jones, who is the Co-Chair for the Utah ERA Coalition. The CP expressed a need for an individual with many contacts and ties to the youth population and experience with organizing, so my

positions as a community leader with the SLC Women's March and as a University of Utah student leader were opportune for the organization. My Faculty Mentor is Professor James Curry, an associate professor and Director of Graduate Studies in the Department of Political Science at the University of Utah. Curry's research focuses on U.S. politics and policymaking, especially legislative politics.

Project Goals & Description

The overall goal of this project is to educate and engage youth about the Equal Rights Amendment through legislative advocacy. In order to do this, the smaller project goals were to create a comprehensive education curriculum in a social justice lens for youth (high school and college), develop toolkits about legislative advocacy for use in preparation for the next state legislative session, and to develop meaningful relationships between the youth and the Community Partner.

First, I started with the education curriculum by creating appealing infographics and compiling these into readable files and posting them on the Community Partner's website. For this, I worked closely with my Community Partner. Second, I started developing the toolkits, including, but not limited to, email/speech templates, general action and resource guides, and checklists for organizing. My FM helped me on effective methods to interact and engage with legislators. After the development

stages (conducted during the summer), with the help of my Community Partner, we reached out to youth across school districts and colleges in Utah with an interest survey as the school year starts to begin around August. Those interested went through the entire semester of educational material and training I've developed beforehand. This segment began with recorded live webinars for youth to attend to learn from the presentations and infographics. Then, the segment shifted over to learning how to take action with the toolkits. There were also be other extraneous events organized by the Utah ERA Coalition leadership that will be incorporated into the semester-long training.

Sustainability

Once the youth education and engagement were completed, the materials would continue to be accessible on the Community Partner's website and in possession of the Community Partner for their use in the future since these are the only pieces they will have to engage youth with. What was learned is also sustained because this newly acquired knowledge and skill sets that I've helped teach will be applied to legislative advocacy for the ERA in the next legislative session and can still be applied to other areas of activism. This increases the influence and popularity of the Utah ERA Coalition, which will help the organization gain more attention and connections. Another byproduct of my project is sustained youth

engagement with the Utah ERA Coalition because the relationships and interests have been built. Future collaborative efforts and opportunities will build upon this foundation I've laid out in my project for both groups that drive ERA ratification efforts in Utah further.

Assessment Methods & Expected Impact

This project was assessed in quantitative and qualitative assessments. I measured the quantity of my effort through time put in, number of connections made with youth, and number of educational resources posted on the Utah ERA Coalition website. I also measured the quality of my effort by examining whether youth have engaged with the curriculum and toolkits, whether the youth and the Community Partner enjoyed using these, and whether youth's knowledge of the ERA and skills have changed. I measured the effect of my project by investigating if youth's knowledge and skills have changed, if their interest is expressed to continue involvement, and if the organization's efficacies to educate youth have improved. Taking the responses expressing interest, my Faculty Mentor and I created pretest to ask what knowledge these youth have about the ERA and what skills/experience they currently have with legislative advocacy. Another pre-test asked of the Community Partner's level and efficacies of engagement/involvement with youth through the number of connections and interactions. Then, my

Faculty Mentor and I created post-tests to ask if the material and training had helped their knowledge and skills. Another post-test asked if the Community Partner liked it as well, if they saw an increase in youth engagement and improved efficacies, and whether they'll continue to use my material.

The expected impacts here were increased knowledge, connections, skills, and engagement youth will gain to further ERA legislative advocacy, and increased youth base and influence for the Utah ERA Coalition. The impacts were made conceivable and attainable by how my project established a baseline to start from and the infrastructure to coordinate collaboration between both groups. The overall long-term impact was that there is more of an organized, effective legislative advocacy for the ERA ratification in Utah from the efforts of youth activists.

Works Cited

Arnold, R. D. (1990). The Logic of Congressional Action. Yale University Press.

Broockman, D. (2013). Black Politicians Are More Intrinsically Motivated to Advance Blacks' Interests: A Field Experiment Manipulating Political Incentives. American Journal of Political Science, 57(3), 521-536. Retrieved April 1, 2021, from http://www.jstor.org/stable/23496636

Cohen, A., & Codrington III, W. U. (2020, January 23). The Equal Rights Amendment Explained. Brennan Center for Justice.

https://www.brennancenter.org/our-work/research-reports/equal-rights-amendment-explained

Earl, J., Maher, T. V., & Elliott, T. (2017). Youth, activism, and social movements. Sociology Compass, 11(4), 1–14. https://doi.org/10.1111/soc4.12465

Fisher, D. R. (2012). Youth Political Participation: Bridging Activism and Electoral Politics. Annual Review of Sociology, 38, 119–137. https://doi.org/10.1146/annurev-soc-071811-145439

Gay, C. (2004). Putting Race in Context: Identifying the Environmental Determinants of Black Racial Attitudes. American Political Science Review, 98(4), 547–562. https://doi.org/10.1017/S0003055404041346

Holman, M. R., & Mahoney, A. (2018). Stop, Collaborate, and Listen: Women's Collaboration in US State Legislatures. Legislative Studies Quarterly, 43(2), 179–206. https://doi.org/10.1111/lsq.12199

Mayhew, D. R. (1974). Congress: The Electoral Connection. Yale University Press.

Minta, M. D., & Brown, N. E. (2014). INTERSECTING INTERESTS: Gender, Race, and Congressional Attention to Women's Issues. Du Bois Review: Social Science Research on Race, 11(2), 253–272. https://doi.org/10.1017/s1742058x14000186

Rosenstone, S. J., & Hansen, J. M. (2010). Participation. In K. Kollman (Ed.), Readings in American Politics: Analysis and Perspectives (3rd ed., pp. 399–415). W. W. Norton & Company.

Why We Need the Equal Rights Amendment. (2018). Equal Rights Amendment. https://www.equalrightsamendment.org/why

Wiener, E. (2020). Getting a High Heel in the Door: An Experiment on State Legislator Responsiveness to Women's

Issue   Lobbying.   Political   Research   Quarterly,   1–15.
https://doi.org/10.1177/1065912920939186

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120. **Investigating the Perseverance and Bias Blind Spot Relationship**

Jordan Davidson and
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Faculty Mentor: Frank Drews (Psychology, University of Utah)

ABSTRACTAlthough cognitive bias is easily detectable in the judgments and decision making of other people, we often fail to see its influence in our own thoughts and behavior. This "bias blind spot" is present in most individuals, but its magnitude differs from person to person. To better understand individual differences in susceptibility, we examined the relationship between bias blind spot and perseverance. To measure perseverance, participants were asked to complete as many easy or difficult mazes as they were able to do. Bias blind spot was measured using a validated scale developed by Scopelliti et al. (2015). Bias blind spot susceptibility was

compared to time spent on the mazes and number of mazes completed. ANCOVA results revealed a significant finding between bias blind spot and measures of perseverance on difficult mazes, suggesting a relationship exists for bias blind spot and perseverance on challenging tasks. A second study will measure differences in perseverant behavior when feedback is introduced that compares the participant's number of mazes completed to the average number completed by peers.

INTRODUCTIONIt is well understood that cognitive biases influence judgment and decision making. This has been long demonstrated by psychologists (Kahneman et al., 1982; Nisbett & Ross, 1980) as well as through casual observation by the average person. It is generally well understood that people can deviate from rationality when they are unduly influenced by factors such as political ideology, personal beliefs, and the desire to make a good impression.

However, while cognitive biases are easy to intuit and detect in the motivations of the people around us, the same evidence of bias is no longer obviously apparent when one reflects on their own judgment and decision making. Engaging in meta-cognitive considerations, people tend to believe that their own perspective is uniquely objective and that those who disagree with their assertions are unilaterally biased, exhibiting a sort of "naïve realism" (Robinson et al., 1995).

This asymmetrical ability to perceive cognitive bias in the self versus others is known as the bias blind spot, and appears to be present in the majority of people (Pronin et al., 2002; 2004).

While the bias blind spot is pervasive, there are individual differences to what extent a person displays bias blind spot, i.e.,

some individuals with a bias blind spot believe more strongly that they are less biased than others while some believe they are only a little less biased than average (Scopelliti et al., 2015). While these individual differences are apparent throughout much of the research on bias blind spot, there is a perplexing lack of correlations between bias blind spot and various measures of cognitive sophistication (i.e., thinking dispositions and cognitive abilities) (West et al., 2012), personality, and self-evaluative measures (Scopelliti et al., 2015). Possibly motivated by the lack of correlational findings, research has focused more on the cognitive processes underlying bias blind spot.

The cognitive mechanisms that guide bias blind spot are not thought to be unidimensional. Instead, several contributors to its existence are discussed in the literature. Primarily, bias blind spot is believed to come from the "introspection illusion," or a tendency to believe our own intuitions reveal objective insight for ourselves while others' intuitions are riddled with bias (Pronin & Kugler, 2007). This belief leads to the aforementioned naïve realism bias, another potential process behind bias blind spot. Similarly, research has also shown that people tend to overemphasize their own intentions when self-evaluating while simultaneously ignoring the intentions of others when evaluating them (Kruger & Gilovich, 2004). While naïve realism and the introspection illusion are largely considered to be the main motivator behind bias blind spot, there is still the possibility that this metabias could also be—at least partially—explained in self-enhancing motivational terms (Pronin et al., 2002; West et al., 2012).

There are a number of ways that people exhibit other "positive illusions," or unrealistically optimistic views of the self (Taylor & Brown, 1988). However, these illusions are not always detrimental, and have been argued by Taylor & Brown

to foster positive mental health outcomes. Additionally, positive illusions may be associated with increased ambition and perseverance (Johnson et al., 2013). As bias blind spot could be considered as a type of positive illusion, it stands to reason that it may be associated with an increased ability to persevere.

In research conducted by Styk and Klinkosz (2020), perseverance is believed to be a product of individual dispositions and situational factors. Furthermore, there are individual differences in

levels of perseverance. While the operational definition of perseverance is not as well-defined as bias blind spot within current literature, Styk and Klinkosz described perseverance as an ability to pursue a goal by overcoming a desire to give up even in the presence of distractions. This definition of perseverance was adopted for the current study.

In the present research, we explore perseverance as a potential motivator for the bias blind spot. While previous research has largely investigated the consequences (Scopelliti et al., 2015) and cognitive mechanisms (Mandel et al., 2022; Pronin et al., 2007; West et al., 2015) behind bias blind spot, research into its potential advantages has been less investigated.

METHOD

ParticipantsIn exchange for course credit, 107 undergraduates from a large university participated in this Institutional Review Board approved study. Because of either technical difficulties (3) or violation of prohibited cell phone usage (3), 6 participants were excluded from the final data set. The participants (59 women, 34 men, 8 other/non-binary) were mostly younger than 20 years old (72.3%) or between 20-22 years of age (22.8%). The remaining participants (4.9%) were

older than 22 years old. The sample comprised 1 participant who identified as American Indian/Alaskan Native/ Indigenous, 17 as Asian/Asian American, 1 as Black/African American, 70 as Caucasian/White, 22 as Hispanic or Latino/a, 1 as some other race/ethnicity, and 1 chose not to respond. There was some overlap between the various racial/ethnic identifications where 13 participants identified as multiple races or ethnicities.

Materials*Bias Blind Spot.* The bias blind spot scale developed by Scopelliti et al. (2015) was used verbatim for the study to assess the magnitude of bias blind spot in participants. The scale comprised 14 different common cognitive biases. A brief description of each bias was given, followed by two questions pertaining to the bias in question. The first question asked, "to what extent are **you** likely to show this tendency?" on a 7-point Likert scale (1 = not at all likely, 7 = very highly likely), followed immediately by a similar question asking, "to what extent do you believe the **average University of Utah student** is likely to show this tendency?" on the same 7- point Likert scale.

For each of the 14 cognitive biases, the rating given to the self was subtracted from the rating given to the average University of Utah student to create a "bias score" for each participant. A positive bias score was indicative of bias blind spot for that bias. For example, if a participant had a bias score of 1 for the action/inaction bias, this meant they rated other students as being 1 point more susceptible than themselves to committing action/inaction bias. A "bias blind spot score" then aggregated each bias score for the participant. This worked much the same way as the individual biases: if a participant had a bias blind spot score of 1, that meant that— across all

14 biases described—they believed other students to be 1 point more susceptible on average to cognitive bias.

Perseverance. In order to examine perseverance, participants were asked to complete randomly generated mazes on a computer (see Figure 1).
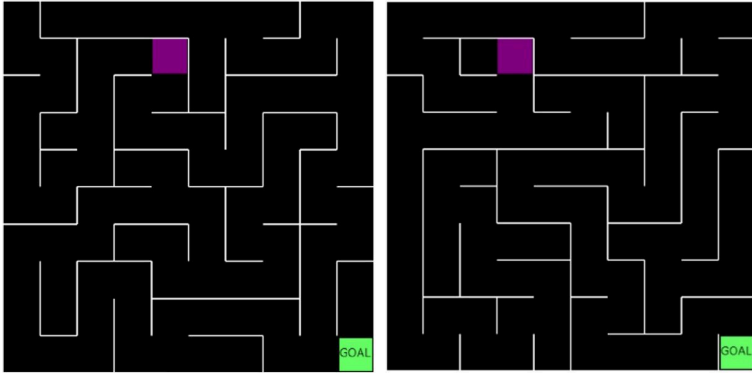
Figure 1. Examples of an easy (left) or difficult (right) maze.

Half of the participants were given "easy" mazes while half were given "difficult" mazes. Easy mazes had one direct route to the goal while difficult mazes generated multiple pathways and dead ends. The different level of mazes were created to see if there was a difference in performance for a sustained monotonous endeavor through the easy mazes—more in line with how Styk and Klinkosz measured perseverance (2020)—and sustained cognitive involvement as a form of perseverance in the form of the difficult mazes.

The maze task was adapted from Styk & Klinkosz (2020) where mazes were used to measure perseverance. However, unlike Styk and Klinkosz's study that used a publicly accessible website, a private website was created for the sole purpose of the study. The mazes were written in JavaScript for easier web integration, and featured keyboard movement instead of

the mouse movement that Styk and Klinkosz's mazes utilized. Three main data points were also recorded for each participant to measure their individual levels of perseverance: (1) the number of mazes completed before ending the study, (2) the total amount of time spent on the maze portion of the study, and (3) time on task, i.e., how much of their total time was spent actively solving mazes (after accounting for load time of each maze and delays in moving through the maze).

Procedure

Research assistants (RAs) were trained to avoid using the word "bias" with participants at all stages of the experiment until debrief. Previous research has shown that using the negatively connotated word "bias" can incidentally bias participants into answering questions in a way that makes them appear more objective (Pronin et al., 2002; Scopelliti et al., 2015). A more neutral term, "cognitive tendency," was used instead in both verbal and written instructions. Participants were randomly assigned to easy or difficult mazes before they arrived.

After consent was obtained, participants were instructed to complete a survey on Qualtrics in a separate, private room. The survey contained the 14-point bias blind spot scale and demographic questions. A randomly generated ID number was created by Qualtrics at the end of the survey. This anonymized number identifying participant information was combined with the maze data while simultaneously allowing survey and maze data to be linked together for data analysis. The participants were instructed to alert the RA after completion of the survey to move on to the second part of the study. The RA would then fill out a form that generated easy or difficult mazes depending on the participant's predetermined assignment. RAs

would deliver verbal instructions from a script to standardize how participants were instructed to complete the maze portion of the task. Because we were concerned with how perseverant the participants were, instructions were intentionally vague when asking the participants to complete mazes. Participants were asked to complete as many mazes "as they were able to do." This gave them the opportunity to end the task based on their own assessment of when they felt unable to continue, thus measuring their perseverance limit for the maze task. Once they had completed as many mazes as they were able to do, they were instructed to click a Quit button that was able to be clicked at any time while doing mazes. Finally, the participants answered two questions related to difficulty and two questions related to the enjoyability of the mazes. Participants were then debriefed and given the opportunity to ask questions. Course credit was given for their participation.

RESULTS As expected, the bias blind spot was successfully replicated: participants rated themselves as less susceptible (M=3.72) than other University of Utah students (M=4.64) to the biases, $t(100)$=13.154, p<.001. Asymmetrical ratings between self and other were noticeable and statistically significant for almost all of the 14 individual biases (all ps<.001), with a notable exception for the ostrich effect, which did not reach statistical significance between ratings of self and other (p=.134). Since the ostrich effect was not significant, we

eliminated this bias from our calculation of bias blind spot and data analysis.

There was also one apparent difference between measures of performance on easy versus difficult mazes. Participants completed more easy mazes (*M*=92.67) than participants who completed difficult mazes (*M*=65.76), *t*(100)=2.908, *p*=.004. However, there were no statistically significant differences in total time spent on mazes between the two difficulties (*p*=.882), nor was there a significant difference between the two difficulties of mazes in time on task (*p*=.086).

An ANCOVA was conducted to determine significant differences between maze difficulty level on number of mazes completed, total time spent on the maze task, and time on task while controlling for bias blind spot. The effect of maze difficulty on the number of mazes completed by each participant (*F(1,100)*=4.961, *p*=.028), total time spent on the mazes (*F(1,100)*=5.154, *p*=.025), and time on task (*F(1,100)*=4.994, *p*=.028) were all significant. This revealed that bias blind spot and maze difficulty were significant predictors of all three measures of perseverance.

Further analysis showed that bias blind spot score was only significantly correlated with measures of perseverance on the difficult mazes. Bias blind spot score and number of mazes completed were found to be moderately and positively correlated on difficult mazes, *r*(100)=.387, *p*=.005, while showing a nonsignificant weak, negative correlation on easier mazes, *r*(100)=-.096, *p*=.505. A similar effect was discovered when examining time on task. On difficult mazes, a positive moderate correlation was found to be significant between bias blind spot score and time on task, *r*(100)=.433, *p*=.002, while remaining nonsignificant for easier mazes, *r*(100)=-.005, *p*=.997. Bias blind spot was also moderately and

positively correlated with total time spent on difficult mazes, $r(100)=.41$, $p=.003$ and not significantly correlated on easier mazes, $r(100)=.005$, $p=.974$ (see Figure 2).
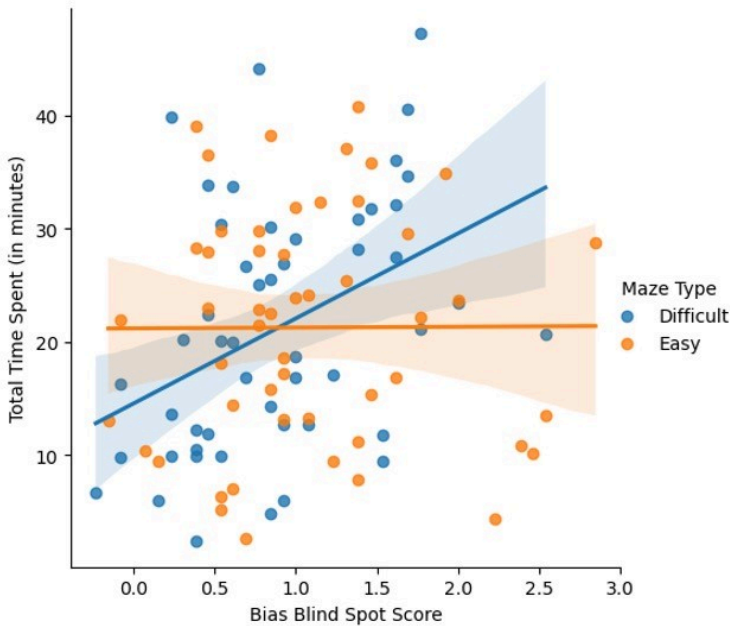
Figure 2. Scatterplot showing the relationship between bias blind spot score and total time spent on the maze task for each participant, split by maze difficulty. The other two measures of perseverance—time on task and number of mazes completed—had a similar relationship to bias blind spot score.

DISCUSSION

Since ANCOVA results were significant for all three measures of perseverance when controlling for bias blind spot, it can be inferred that individual differences in bias blind spot affect performance and behavior on tasks requiring perseverance. Participants who displayed a higher bias blind

spot score—and therefore, a more positive view of the self in terms of rational decision making—tended to persevere on more difficult tasks. This finding could lend insight into why the bias blind spot may, in turn, be a persistent feature of human cognition: a more positive view of one's rational decision making could motivate one to persevere. While the bias blind spot certainly has negative consequences (Scopelliti et al., 2015), there could be more personally beneficial motivators for its existence.

It is notable that the relationship between bias blind spot and measures of perseverance were only significant for the more difficult mazes, and not significant whatsoever for easier mazes. In effect, the easier mazes served as a control group for this study. It was only when participants needed to persevere on more cognitively involved tasks that bias blind spot contributed to a participants' level of perseverance, while perseverant behavior on more mindless tasks is unaffected by a person's magnitude of bias blind spot. It can be deduced that bias blind spot may benefit an individual's perseverance only when they must persevere on difficult or challenging tasks.

Data collection for a second study is currently ongoing, in which participants are shown feedback on performance. Data from this study will be shown to the participant, namely how many mazes the average participant completed. A counter is displayed to the side of the maze that shows in real time how many mazes the participant has completed and also a true average number of mazes other students have completed, based on the current study. This second study is designed to see

how much a direct self/other comparison will affect perseverant behavior in participants depending on their magnitude of bias blind spot. If measures of perseverance are affected differently by this feedback, it could indicate that perseverant behavior in those with a higher bias blind spot is grounded in a need to be better than others, rather than a need to look at oneself positively. This would indicate whether bias blind spot manifests more from an inward introspection of the self or a more outward need to be unique or better-than-average compared to peers.

REFERENCESJohnson, D. D. P., Blumstein, D. T., Fowler, J. H., & Haselton, M. G. (2013). The evolution of error: Error management, cognitive constraints, and adaptive decision-making biases. *Trends in Ecology and Evolution, 28*(8), 474-481. https://doi.org/10.1016/j.tree.2013.05.014

Kahneman, D., Slovic, P., & Tversky, A. (Eds.). (1982). *Judgment under uncertainty: Heuristics and biases.* Cambridge University Press.

Kruger, J., & Gilovich, T. (2004). Actions, intentions, and self-assessment: The road to self- enhancement is paved with good intentions. *Personality and Psychology Bulletin*, *30*(3), 328-339. https://doi.org/10.1177/0146167203259932

Mandel, D. R., Collins, R. N., Walker, A. C., Fugelsang, J. A., & Risko, E. F. (2022).

Hypothesized drivers of the bias blind spot—cognitive sophistication, introspection bias, and conversational processes. *Judgment and Decision Making*, *17*(6), 1392-1421. https://doi.org/10.1017/S1930297500009475

Nisbett, R. E. & Ross, L. (1980). *Human Inference: Strategies and Shortcomings of Social Judgment.* Englewood Cliffs, NJ, USA: Prentice-Hall.

Pronin, E., Lin, D. Y., & Ross, L. (2002). The bias blind spot: Perceptions of bias in self versus others. *Personality and Social Psychology Bulletin, 28*(3), 369-381. https://doi.org/10.1177/0146167202286008

Pronin, E., Gilovich, T., & Ross, L. (2004). Objectivity in the Eye of the Beholder: Divergent Perceptions of Bias in Self Versus Others. *Psychological Review, 111*(3), 781-799. https://doi.org/10.1037/0033-295X.111.3.781

Pronin, E. & Kugler, M. (2007). Valuing thoughts, ignoring behavior: The introspection illusion as a source of the bias blind spot. *Journal of Experimental Social Psychology, 43*(2007), 565-578. https://doi.org/10.1016/j.jesp.2006.05.011

Robinson, R. J., Keltner, D., Ward, A., & Ross, L. (1995). Actual versus assumed differences in construal: "Naive realism" in intergroup perception and conflict. *Journal of Personality and Social Psychology, 68*(3), 404-417. https://doi.org/10.1037/0022-3514.68.3.404

Scopelliti, I., Morewedge, C. K., McCormick, E., Min, H. L., Lebrecht, S., & Kassam, K. S. (2015). Bias blind spot: Structure, measurement, and consequences. *Management Science, 61*(10), 2468-2486. https://doi.org/10.1287/mnsc.2014.2096

Styk, W., & Klinkosz, W. (2020). The maze test: A computer tool for testing perseverance.

Psychology Research and Behavior Management, 2020(13), 1277-1288. https://doi.org/10.2147/PRBM.S271558

Taylor, S. E. & Brown, J. D. (1988). Illusion and well-being: A social psychological perspective on mental health. *Psychological Bulletin, 103*(2), 193-210. https://doi.org/10.1037/0033- 2909.103.2.193

West, R. F., Meserve, R. J., & Stanovich, K. E. (2012). Cognitive sophistication does not attenuate the bias blind spot. *Journal of Personality and Social Psychology*, 103(3), 506-

519.   https://doi.org/10.1037/a0028857

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121. **An Educational Intervention for Congenital Cytomegalovirus: Pre- and Post-Education Knowledge and Behaviors**

Shaistah Din; Jessica Sharma; Abel Chavez; and Marissa Diener (Family Consumer Studies)

Faculty Mentor: Marissa Diener (Family and Consumer Studies, University of Utah)

Congenital Cytomegalovirus (cCMV) is the leading cause of non-genetic sensorineural hearing loss (SNHL) in the United States (Lanzieri et al., 2017). If present at birth, it can result in hearing loss most commonly, but also permanent neurocognitive disability and musculoskeletal impairment in children (Pesch et al., 2021). It has significantly higher rates of infection than diseases such as down syndrome or spina bifida, but very low levels of awareness among the public (Diener et al., 2020). Our study involved investigating the impact of a CMV education intervention on the knowledge, attitudes, and behaviors of pregnant women towards cCMV. As part of the educational intervention, 107 pregnant women completed pre-education and posteducation surveys that were randomized between a print and video format. Some women received the survey and educational materials via email, while others received the materials in-person at the clinic. The pre and post surveys occurred 8 to 10 weeks apart. Preliminary results were analyzed (see figures below), and they portrayed that women changed their hygiene behaviors after education. Women also viewed the educational materials favorably and their knowledge about the virus shows an increase after education as well. There was not a difference noted between remote and in person education formats. The project results portray the significance of educational intervention in changing behaviors and attitudes. The long-term goal is to help scale up educational efforts to prevent CMV and to survey more diverse populations.

Diener, M L., et al. (2020). A Cross-Sectional Study of Caregiver Perceptions of Congenital Cytomegalovirus Infection: Knowledge and Attitudes about Screening. The

Journal of Pediatrics, Feb 139(2): e20160789. Doi: 10.1542/peds.2016-0789

Lanzieri, T. M., Chung, W., Flores, M., Blum, P., Caviness, A. C., Bialek, S. R., ... & Congenital Cytomegalovirus Longitudinal Study Group. (2017). Hearing loss in children with asymptomatic congenital cytomegalovirus infection. Pediatrics, 139(3).

Pesch MH, Saunders NA, Abdelnabi S. (2021). Cytomegalovirus Infection in Pregnancy: Prevention, Presentation, Management and Neonatal Outcomes. J Midwifery Womens Health. May;66(3):397-402. doi: 10.1111/jmwh.13228. Epub 2021 May 24. PMID: 34031974.

Figure 1: Knowledge of cCMV Before and After cCMV Education
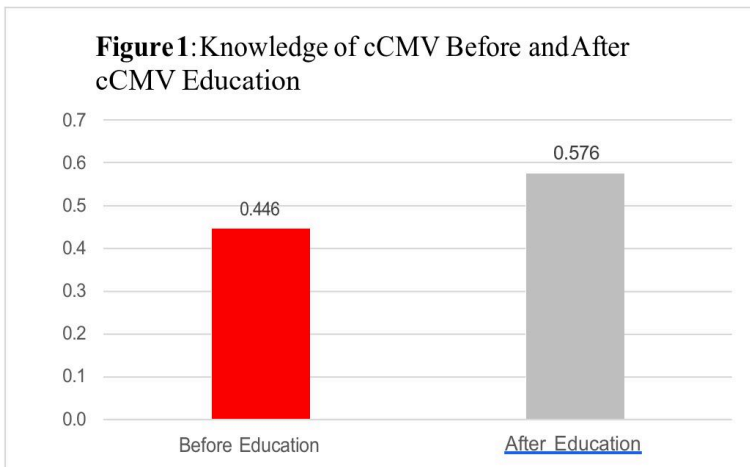
Figure 2: Percentage of Women who had heard of CMV - Pre and Post Education
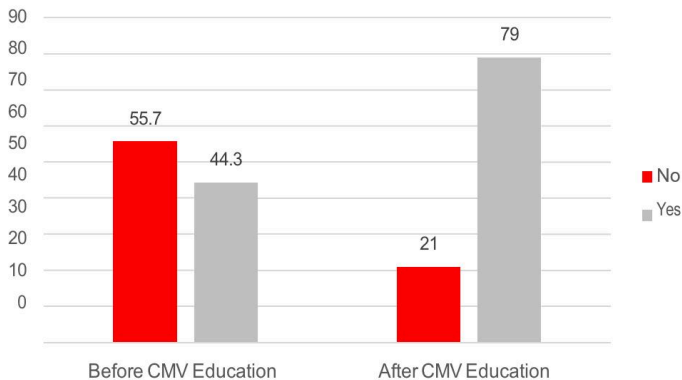
Figure 3: The Impact of Type of Education on cCMV Knowledge Scores
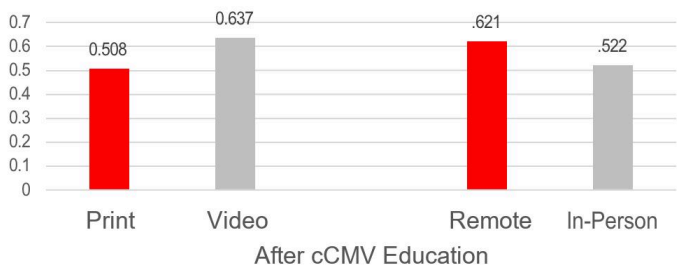
Figure 4: Correlation of factors related to knowledge - Pre and Post CMV education
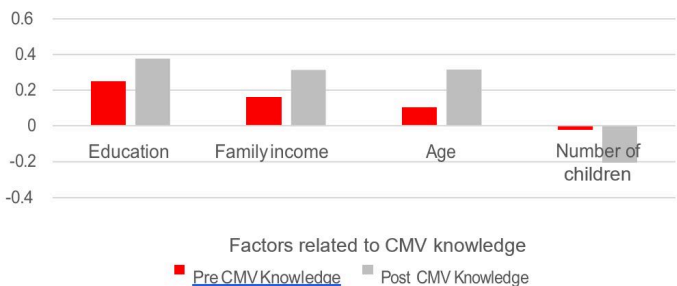
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122. **Research Reflection by Shaistah Din**

Shaistah Din

Faculty Mentor: Marissa Diener (Family and Consumer Studies, University of Utah)

The opportunity to participate in this clinical research study has been one of the most impactful experiences of my undergraduate career. Not only was I able to participate in research that was of interest to me and directly related to my passions, but I have also gained an amazing group of colleagues along the way through Dr. Diener and my fellow research peers. Moreover, I am a double major in biology & health, society, and policy. Overlapping these two fields has enriched my undergraduate experience and as a prospective medical student, I have learned so much along the way. As an interdisciplinary student, I have been able to gain multiple perspectives that I know will be of benefit to me in the future as a healthcare worker. The research I am a part of directly relates

to the public health field and has implications in the medical arena as well. My future goal is to be a physician that advocates for women's health and this research is a crucial aspect of spreading awareness and knowledge about a significant prenatal disease. I am honored to be able to be a part of this study and am looking forward to the future work that will be done in this field.

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123.   **Genomic insights into the evolution of cytokine tissue specificity across primates**

Aidyn Eldredge and
Timothy Webster
(Anthropology)

Faculty Mentor: Timothy Webster (Anthropology)

Cytokines are immune glycoproteins released in a hormone-like manner to promote the regulation of the inflammatory response upon pathogen recognition. As such, their dysregulation has been linked to several physiological disorders, including autoimmune diseases. While their medical importance has been well-established, far less is known about

their evolutionary patterns. In this study, we used RNA-seq data from heart, kidney, liver, and lung tissue samples collected from the same individuals of three primate species: human (Homo sapiens), chimpanzee (Pan troglodytes), and rhesus macaque (Macaca mulatta). We measured the expression of 314 previously annotated cytokine genes and quantified tissue specificity of expression using the tau metric. We found that rhesus macaques (τmean=0.840; τmedian=0.886) demonstrated, overall, a greater degree of tissue specificity than humans (τmean=0.807; τmedian=0.861) and chimpanzees (τmean=0.790; τmedian=0.813) (Urhesus-human=64287.5, p=0.0297; Urhesus-chimp=55322, p=0.00042), while humans and chimpanzees did not differ significantly (Uhuman-chimp=112288, p=0.122). Our ongoing analyses build on these results by investigating the degree to which tissue-specific cytokines maintain their specificity across lineages, provide insight into the evolutionary patterns of individual cytokine genes. Together, these results will contribute to our understanding of the evolution of primate immune responses.

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124. **Research Reflection by Aidyn Eldredge**

Aidyn Eldredge

Faculty Mentor: Timothy Webster (Anthropology)

Working with my faculty mentor on this project was a thoroughly enjoyable experience. My participation in undergraduate research gave me a valuable insight into the world of anthropology research. During this experience, I applied my classroom knowledge to real-world problems and gained practical experience in the analysis of genomic data. Investigating the correlation between genetics and human immune function was particularly fascinating for me, as it aligns with my interest in pursuing a career in medicine. Through this research, I was able to develop critical thinking and problem-solving skills, as I conducted a comprehensive literature review, created a pipeline, and analyzed the data. I am confident that these skills will be useful as I continue my education.

About the Author

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125. **Evaluating the Efficacy of Asynchronous and Synchronous Problem-Solving Teleconsultation with Teachers who Serve Rural Students with Disabilities**

Mickenzie Fleming

Faculty Mentors: Aaron Fischer and Shengtian Wu (Psychology, University of Utah)

Abstract

Special education services are available to help children who are classified with disabilities including Autism, Intellectual Disability, and Developmental Disability. Generally, these additional services include environmental accommodations and individualized support within smaller class sizes and in the form of one-on-one or small group interactions with teachers or paraprofessional(s). Although, in-person consultations are not always plausible due to time constraints or geographical distances. Problem-solving teleconsultation is an evidence-based strategy and is most effective and commonly used in schools (King et al., 2021). Through this approach, the consultant conducts a Functional Behavioral Assessment (FBA) to understand the underlying reason why the behaviors are occurring. The assessment allows the teleconsultants to accurately develop and implement an individualized and function-based positive behavior plan, and other class-wide interventions, to assist educators as they provide instruction and behavior support.

IntroductionIn schools, the prevalence of students endorsing mental and behavioral health concerns continues to increase (National Center for Educational Statistics, 2020). Students with disabilities such as Autism, Intellectual

Disability, and Developmental Disability need individualized support. Special education services allow teachers to focus on the individual needs of each of their students, with more nuance and fewer distractions for the student. Despite these educational efforts, some students engage in disruptive behavior (e.g., property destruction, elopement, physical aggression) and require advanced behavior assessment and treatment services to maximize student social emotional, behavioral, and learning outcomes. In these instances, a school-based consultant will often help teachers to develop and implement these advanced strategies with their students while providing ongoing supervision and collaboration.

A better service would be a form of teleconsultation. Teleconsultation is defined as synchronous or asynchronous consultation using communication technology to remove geographical distances (Deldar et al, 2016). Asynchronous communication can happen over a period without an immediate response. No studies have tested the limits of teleconsultation to see which aspects could be conducted solely asynchronously. With more services (e.g., observations, intervention, planning, and coaching) conducted at the convenience of the consultation team, each member can engage when it works in their schedule while remaining responsive. A study comparing teacher's relationship between time management skills and their classroom performance has shown a positive trend (Khan, 2016). The research team will give guidance and feedback to educators of students with disabilities using problem-solving teleconsultation procedures but in a completely asynchronous fashion.

Methods A survey was given to the student's educator to create a functional behavior assessment (FBA) to determine

any targeted behaviors, antecedents, and consequences. The educator will video record the student during an activity and document the student's behaviors every time it was displayed for a baseline measurement. Once this data was collected, the consultant will then record themselves providing a summary of operational definitions and their collections of baseline measurement. Then a behavioral intervention plan (BIP) will be decided for the student.

The next phase includes implementing the agreed-upon BIP and providing training for the educator through performance feedback. BIP components include antecedent interventions and consequence-based interventions. BIP will be implemented every day with the student but only recorded during a ratified time and activity that usually stimulates a response. After recordings are evaluated, the consultant reviews them and the data collected with the educator. This allows for any changes to the BIP. These recordings will happen for about 2 months.

The consultant will begin a follow-up observation four weeks after the end of the asynchronous teleconsultation. The student will be recorded with the educator 2-3 times per week for a total of two weeks without the educator's implementation. The consultant will measure the overall improved student behaviors.

DataOverall Problem Behaviors-Figure 1

Overall Problem Behaviors-Figure 1
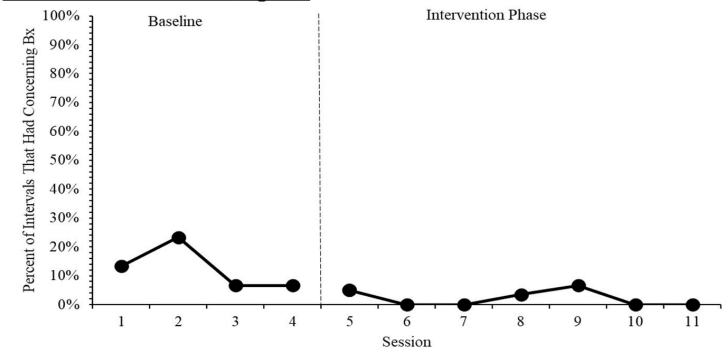
Teacher Report on Self-Stimulus Behaviors-Figure 2

Teacher Report on Self-Stimulus Behaviors-Figure 2
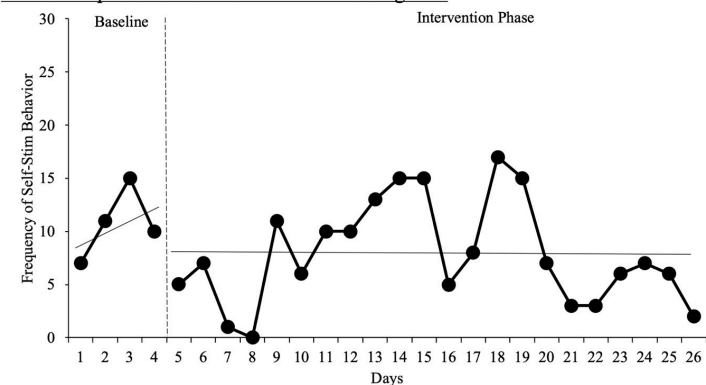
Social validity measurements-Figure 3

Social validity measurements-Figure 3

	Ave Rating
FFTAM	1.92 (SD=0.51; Range=1-3)
CEF	6.83 (SD=0.39; Range=6-7)
BIRS	4.58 (SD=0.58; Range=3-5)

Teacher Perception Through Goal Attainment Scale-Figure 4

Teacher Perception Through Goal Attainment Scale-Figure 4

	Ave Rating
Functional Communication	1.92 of 2 (*SD*=0.51; Range=1-3)
Compliance	6.83 of 7 (*SD*=0.39; Range=6-7)
Socially Appropriate Behaviors	4.58 of 5(*SD*=0.58; Range=3-5)

Discussion

The participant is a 15-year-old, white male. He was referred to the study by his classroom teacher for a functional behavioral assessment (FBA) and a behavior intervention plan (BIP) for his behavioral concerns. The setting took place in a special education classroom. The student's targeted behaviors are non-compliance, inappropriate communication, and self-stimulation. Figure 1 shows a comparison of the student's overall problem behavior of the baseline measurement to his follow-up measurement. This graph shows a declining trend. A similar graph of the frequency of self-stimulation before and after shows a trend that was increasing to a now flat trend. The social validity in figure 3 shows how the educator perceived teleconsultation. It was measured in technology acceptability (FF-TAM), intervention acceptability (BIRS), and how acceptable the process is as a whole (CEF). These numbers indicate a high social validity for all measurements. Figure 4 describes the educator's perceptibility of the student's progress based on functional communication, compliance, and socially appropriate behaviors. This data infers the educator's perception of the student's targeted behavior increased compared to the baseline measurement.

ConclusionIn conclusion, teleconsultation slightly reduced the student's targeted behavior. His overall problem behavior and self-stimulation behaviors both show declining trends. The educator feels as if teleconsultation was technologically acceptable, the intervention was achievable, and the consultation process for educators was also attainable. The educator's perception of the student's improvement of target behaviors increased since the baseline measurement. More data and participants would be needed to provide a general inference.

Bibliography

Delder, K., Bahaadinbeigy, K., Tara, S.M. (2016). Telecommunication and Clinical Decision Making: A Systematic Review. *Acta Inform*

Hilty, D.M., Torous, J., Parish, M.B., et al. (2021, April 9). A Literature Review Comparing Clinicians' Approaches and Skills to In-Person, Synchronous, and Asynchronous Care: Moving Toward Competencies to Ensure Quality Care. *Mary Ann Liebert, Inc.,* https://doi.org/10.1089/tmj.2020.0054

Khan, H.M.A., Farooqi, M.T.K., Atif, K., Faisal, I., (2016). Exploring Relationship of Time Management with Teachers' Performance. *Eric,* 38(2), 249-263. https://eric.ed.gov/?id=EJ1210299

King, H.C., Bloomfield, B.S., Wu, S., Fischer, A.J., (2021). A systematic Review of School Teleconsultation: Implications for Research and Practice. *School Psychology Review.* DOI: 10.1080/2372966X.2021.1894478

National Center for Educational Statistics. (2020). The Condition of Education 2020. *Institute of Education Sciences.* https://nces.ed.gov/pubs2020/2020144.pdf

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126. Research Reflection by Mickenzie Fleming

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I have had a wonderful experience doing research with the U-TTEC lab. I understand the importance of having a great team that works together to promote the project, gain participants, create a reputable experiment, collect data, and execute a research article. I have seen the need for more people in the research field and how it can impact different communities. This experience will continue with me as I go on to graduate school and I hope I can impact others with my future research.

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127. **Analysis of Charcoal Morphometrics from Known Utah Plants May Inform Fire and Vegetation History Reconstructions**

Amy Harvey; Stella Mosher; and Mitchell Power (Geography)

Faculty Mentor: Stella Mosher (Geography, University of Utah)

Abstract

Fire is one of the major forces that influences vegetation type and ecosystem dynamics of an area. Reconstructing past fire regimes can help us understand not only about the type of plants that were once in an area and how they responded to fire, but also how best resource and landscape management practices can be employed to preserve present ecosystems under a changing climate. This research contributes to the reconstruction of a fire history record from Utah. A newly emerging method, the study of charcoal morphology (shape) and morphometry (particle geometries) is increasingly being used to determine the types of vegetation that have burned. Charcoal morphotypes may provide insight into the fuel type burned, and measured geometries produced by the experimental burning of plant material at a range of combustion temperatures may provide insight into fire intensity. In this research, charcoal particles collected from experimental burning of modern plant reference material were analyzed to later be compared to the morphometric analysis of macroscopic charcoal particles from local lake sediments to understand vegetation change around Utah. At each temperature (250℃, 300℃, and 350℃) there was a significant ($p < 0.05$) morphometric difference between each type of plant, so morphometries likely can be used to identify vegetation type of fossil charcoal. Additionally, grass-type vegetation produced charcoal that was significantly elongated compared to woody vegetation. These findings may shed light on metrics of interest to the paleofire community, such as vegetation change, wildfire temperature, and wildfire intensity.

Introduction

Past research has found that measuring aspect ratios, defined as length:width (L:W) ratios of macroscopic charcoal particles, can serve as a proxy for the fuel type burned (Feurdean, 2021;

Vachula et al., 2021). Evidence suggests that some morphologies vary significantly between different plant types, specifically between grassy fuel types, signified by unitless aspect ratios greater than 3.5, and woody fuel types, signified by aspect ratios less than 2.5 (Vachula et al., 2021). Using known, modern vegetation sampled from around Utah will help to better calibrate how charcoal particles are interpreted in the deep time record by determining the shapes and aspect ratios each type of plant tends to create, then exploring whether there are significant differences between plant types or between different tissues from the same plant, such as stems and leaves, and between different combustion temperatures.

The study site chosen was the Wasatch region of Utah. Five common species were collected from Little Cottonwood Canyon: white fir (*Abies concolor*), big sagebrush (*Artemisia tridentata*), juniper (*Juniperus sp.*), limber pine (*Pinus flexilis*), and gambel oak (*Quercus gambelii*). Common reed (*Phragmites australis*) was collected from within the Salt Lake Valley, near a water source in West Jordan. Findings can be used to directly compare to charcoal particles from fire history samples dating back thousands of years and contribute to the developing significance of charcoal morphometry in determining past fire history.

Methods

Abies concolor, Artemisia tridentata, Juniperus sp., Pinus flexilis, Phragmites australis, and *Quercus gambelii* samples were pressed and dried at the Natural History Museum of Utah. One-centimeter sections of both stem and leaf of each species were burned in a muffle furnace for two hours at temperatures of 250℃, 300℃, and 350℃. Samples were also burned at temperatures of 400℃ and 450℃, however insufficient charred material remained for analysis. Most of the plant material

burned at these temperatures turned to ash, suggesting full combustion of most plant material at higher burn temperatures. Charred samples were then processed by lightly crushing material to mimic natural transport and mechanical breakage, and then strained with water through a 125 µm sieve (Feurdean, 2021).

Charcoal fragments were measured using CharTool, a software package that uses machine learning to measure individual charcoal particle geometries through a Zeiss stereomicroscope at the University of Utah Records of Environmental Disturbance Lab. CharTool measures a range of particle geometries, including area, perimeter, roundness, and aspect ratio (L:W) (Snitker, 2020). This research focused on aspect ratio data which were measured comparing leaf and stem (typically, woody) tissue type for each species at 250℃, 300℃, and 350℃. Charred particles were quantified to about 250-300 pieces per sample, and morphometric data was exported as a .csv file for further analysis of species, tissue type, and temperature. Aspect ratio findings were then analyzed in R.

A novel technique using tangential flash macrophotography, developed at the Natural History Museum of Utah, was used to take high-resolution surface morphology photographs of select experimentally burned charcoal. This technique takes a series of images, which are then stacked, resulting in a limited depth of field but highlighted surface morphology. A Canon R5 mirrorless camera with a 65mm macro lens set at 5X magnification provided a field-of-view covering 7.2 mm by 4.8 mm with an effective magnification of 400X.

Results and Discussion

Experimental Burning and Morphometric Analyses

While measuring morphometrics with CharTool, typical

shapes of each species quickly became apparent. For example, *Pinus flexilis* charcoal particles were typically slightly elongated and sometimes porous, *Phragmites australis* particles were extremely elongated and "stick"-like, and *Artemisia tridentata* particles were typically square and irregular block-like shapes. One weakness of the program is that it can only calculate geometries by tracing the perimeter of the charcoal particle, so nuances such as pores in the charred material or 3-dimensional shapes are not taken into account, although they may be meaningful indicators of some types of plant material.

Phragmites australis charcoal tended to have more variation within itself, likely because there were many long, curved, string-like pieces that may have skewed the data, and an analysis excluding outliers may provide more insight. *Quercus gambelii*, *Pinus flexilis*, and *Abies concolor* typically produced blocky charcoal with very uniform aspect ratios, leading to the conclusion that woody species often have low, uniform aspect ratios.

Charcoal aspect ratios analyzed for statistical significance at 250℃ (Figure 1), 300℃ (Figure 2), and 350℃ (Figure 3) reveal intriguing trends. The most variation in aspect ratio among all plants was when burned at 300℃ (Figure 2), whereas at 250℃ (Figure 1) and 350℃ (Figure 3) *Phragmites australis* was the main source of variation. It is important to note that variation in aspect ratio is highly significant (ANOVA, $p < 0.05$) at each temperature between each type of plant, and between tissue types at both 250℃ and 300℃. Variation in aspect ratio was not significant between tissue types at 350℃ (ANOVA, $p = 0.982$). Because of this overall statistical significance, morphometries can likely be used as a calibration tool for identifying vegetation type from fossil charcoal.

Figure   1.   Experimentally   charred   morphometric measurements for Utah plants burned at 250℃.
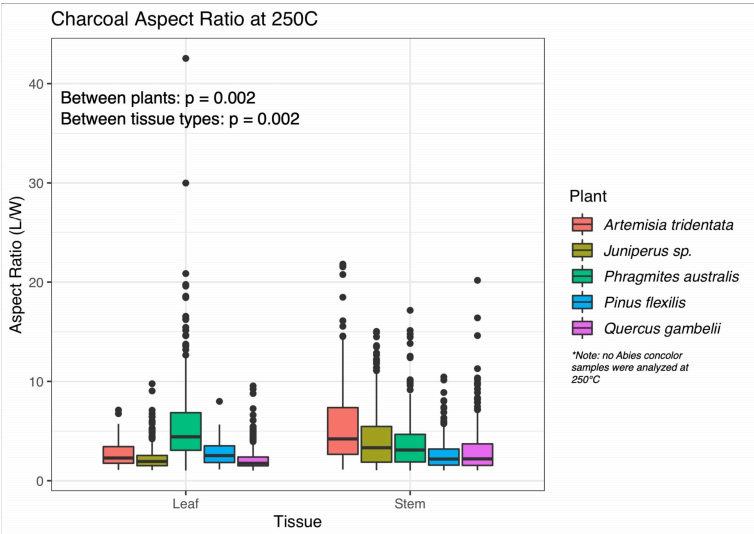
Figure   2.   Experimentally   charred   morphometric measurements for Utah plants burned at 300℃.
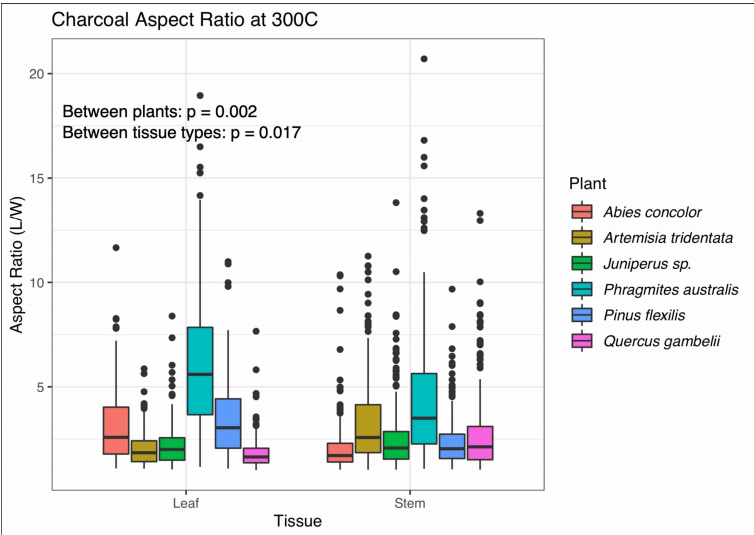
Figure 3. Experimentally charred morphometric measurements for Utah plants burned at 350℃.
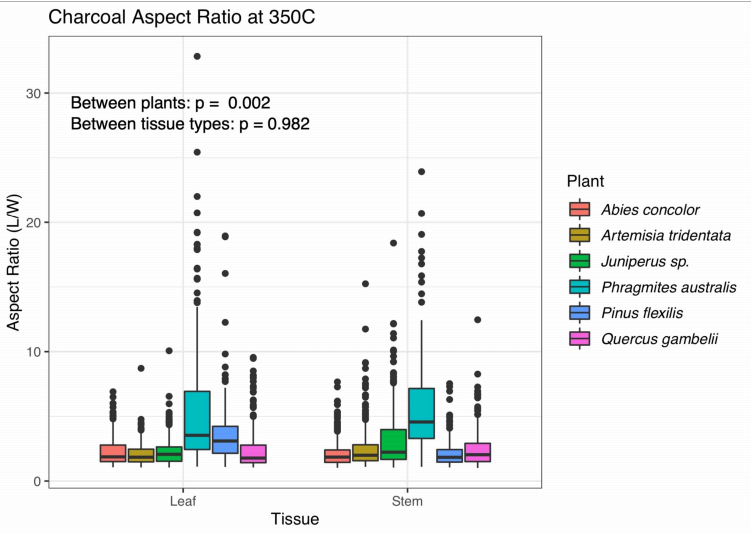
High-resolution charcoal photography

The tangential flash macrophotography pictures captured at the Natural History Museum of Utah (Figure 4) show clear images that exemplify typical morphologies of *Artemisia tridentata*, *Pinus flexilis*, and *Phragmites australis*. CharTool measurement results align with these photographs; for example, *Phragmites australis* charcoal is elongated, showing a higher aspect ratio, and *Artemisia tridentata* and *Pinus flexilis* charcoal pieces are more blocky and round, showing a lower aspect ratio. These photographs also display pores, 3-dimensional features, and lighter areas that did not completely char, something that CharTool analysis does not capture.

Figure 4. Photographs taken at the Natural History Museum of Utah of charcoal pieces of both stem and leaf of *Artemisia*

tridentata, *Pinus flexilis*, and *Phragmites australis* burned at 350℃.
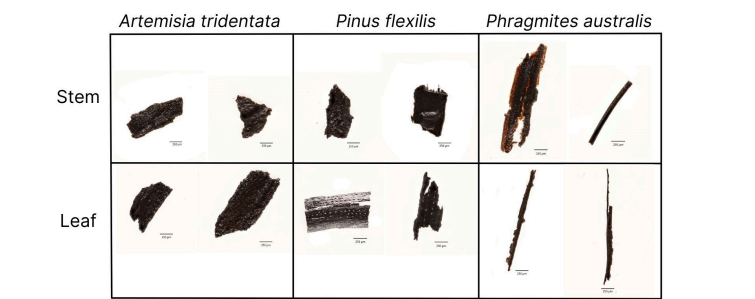
Conclusion

This work suggests that significant differences may exist between plants and tissues at different temperatures which could help us understand what was burning when reconstructing fire histories. If paired with pollen analyses, this information would bolster interpretations of vegetation change over time. To better understand what charcoal morphometrics can tell us about the fire history of an area, the methodology described needs to be continually tested for other species, other temperatures, and other plant tissue types and compared with fossil charcoal found in sediment cores.

References

Feurdean, A. (2021). Experimental production of charcoal morphologies to discriminate fuel source and fire type in the Siberian taiga. *Biogeosciences*.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/

Snitker, G. (2020). A Charcoal Quantification Tool

(CharTool): A Suite of Open-source Tools for Quantifying Charcoal Fragments and Sediment Properties in Archaeological and Paleoecological Analysis. EBL 11, 103-15. https://doi.org/10.14237ebl.11.1.2020.1653

Vachula, R. S., Sae-Lim, J., & Li, R. (2021). A critical appraisal of charcoal morphometry as a paleofire fuel type proxy. *Quaternary Science Reviews.*

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128. Research Reflection by Amy Harvey

Amy Harvey

Faculty Mentor: Stella Mosher (Geography, University of Utah)

Having the opportunity to participate in research has greatly enhanced by undergraduate education. I have gained laboratory skills and experience writing professional academic papers, as well as presented in a research conference. Conducting my own project with the help of my mentor and the amazing faculty I have been able to work with throughout this research experience have made my final year as an undergraduate student more fulfilling and will be very valuable in the future as I further my education and career.

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129. **Religious Trauma Effects on LGBTQ+ Individuals in the LDS Context**

Kathryn Howard

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Abstract

In recent years, there has been more discussion around the trauma and trauma responses that come from being a queer individual (one who identifies with members of the LGBTQIA+ community) inside a high-demand religion that does not affirm existence or behaviors of LGBTQ+ individuals. Emerging literature suggests that existing in an

environment where, as a queer individual, one inherently does not feel respected or safe can lead to depressive, CPTSD, and scrupulosity symptoms, as well as significantly detract from feelings of social safety (Christensen, 2022). Lower sense of self-worth, relationship conflicts with family, the church, and a higher power, depression, and in severe cases, suicide, can all be consequences of existing in these kind of environments as a queer person (Bradshaw, 2015). However, previous research has failed to fully measure the effect that the timing and length of participation and membership in these religious institutions have on the severity and symptomatology of the trauma experienced.

This study is a follow-up from one conducted in December of 2021, which looked specifically at queer individuals in the LDS church (Latter Day Saints). The same participants were contacted to measure any changes from two years ago. In addition to this, the survey was also open to new participants. The new survey included questions that were not asked in the first survey but have proved themselves relevant. This included asking more questions about the amount of time spent in the LDS church and how devoted the individual was during these times. This line of questioning was undertaken so one can see how timing and duration correlates with trauma symptomatology.

There was a total of 1214 respondents, with a

mean age of 29.9 (*SD* = 7.72). In all, 47.8% were assigned male at birth, 52.2% were assigned female, and 17.8 % had a gender identity different from their birth-assigned gender. Regarding church activity, 64% reported that they remained active in the church at least once a week.

Through these questions it was found that the duration spent in the church significantly correlated with rates of OCD symptoms, and lack of social safety for those in their twenties and younger, and that duration spent in the church was significantly positively correlated with depressive symptoms regardless of age.

INTRODUCTION

It is no secret that religion and queerness often do not mesh well together. However, sometimes it is more than just "not getting along." When assessing homonegativity, which is defined as the disapproval of homosexuality, one study found that the biggest predictor of this variable in individuals was their theological orientation (Village & Francis, 2008). Being queer and surrounded by messages that instill negativity about the very core of a person can have tremendous negative impacts on an individual, especially in regards to their mental and emotional health (Page et al., 2013).

High-demand religions are defined as religions that involve large time and resource commitment, stresses scriptural and leadership infallibility, contain a certain level of separation between the

members "worldly people" and have strict, enforced rules and codes, especially those about one's diet, wardrobe, education, sex and reproduction, marriage and relationships, use of technology, language, and social involvement (Myers, 2017). One high-demand religion where this is particularly true is The Church of Jesus Christ of Latter Day Saints (LDS). In some cases, the constant barrage of religious messages can result in the development of religious trauma. Religious trauma is defined as "a group of symptoms that arise in response to traumatic or stressful religious experiences," (Kingdon, n.d.). While this is a very broad definition with many interrelated variables falling under its scope, religious trauma is becoming increasingly prevalent amongst the LGBTQ+ population (Hollier et al., 2022). In 2018 a study showed that the more LGBTQ+ members attend church services, the more mentally unhealthy they were (Finn, 2019).

"Homosexuality is an ugly sin, repugnant to those who find no temptation in it…..All such deviations from normal, proper heterosexual relationships are not merely unnatural but wrong in the sight of God," (Kimball, 1969). This is a direct quote from Spencer W. Kimball, not only a prolific Latter Day Saint Church leader, but the president of the LDS church at the time. With such strong views of condemnation being expressed in these

teachings, it is not surprising to see that religious trauma is more prevalent among queer people.

One potential outcome of religious trauma is Scrupulosity OCD, which is a subtype of OCD in which one develops "religious or moral obsessions" (IOCFD 2010). Most people are familiar with the type of OCD that causes obsessive hand washing as well as numerical obsessions. Clearly, washing your hands is a good thing, but once it crosses a certain threshold, it is no longer helpful but can prove to have negative effects on one's mental and physical health.

One of the best treatments for this is exposure therapy where one is forced to not wash their hands, so they can see that nothing catastrophic happens. This therapeutic process allows the individual to change their relative perspective on reality, helping them embrace the uncertainty of the world.

This situation mirrors a less well-known subtype of OCD. The rigorous hand washing is replaced with praying, repenting, scripture reading, and/or another religious or moral fixation. Such fixations can reach a degree of harmful behavior, just as the aforementioned issue with the hand washing where one finds themselves unable to leave their home, and sometimes, their church (Albińska, 2022). Can doing these activities be a good thing? Absolutely. They can bring peace of mind to people if that's what they believe. But, just like hand washing, it

can reach a point where it is no longer helpful, and that needs to be addressed (McIngvale et al., 2017).

A study was conducted by Fergus and Rowan in 2014, which was inspired by the lack of research in the field of psychology with the scrupulosity subtype of OCD. This disorder is often found to have strong correlations with religious trauma; there is a volatile relationship of uncertainty that is correlated to this disorder. "Difficulties tolerating uncertainty are considered central to anxiety disorders" (Fergus & Rowatt, 2014). For queer individuals in the LDS church, there is a considerable amount of uncertainty. There is uncertainty of whether or not your feelings are valid, how your family will react, how your bishop will react, and the biggest uncertainty of all – no one can truly answer how god will react (Brandley, 2020). This bolsters the idea that queer individuals are more likely to develop scrupulosity and other anxiety disorders when inside the LDS church due to the uncertainty that it creates. This is why many argue for the need to bring further attention to this issue, specifically (Allen et al., 2015).

In most religions, the LDS church being no exception, the idea of God being a perfect being is taught. Because of this, many people are trained to blame themselves when these issues begin persisting, thinking it is because they are not praying enough or not living a fully righteous life. For those in the LGBTQ+ community, this often

turns into the phenomenon of "praying the gay away" that many people go through (Ashworth, 2022). There are countless stories of Mormon kids denying their sexuality and feeling increasing shame as they grew up and discovered where their sexual desires lay. As this shame gets internalized, it starts to spread to every part of one's life. When you start to believe that who you are is simply wrong or a sin, your self-esteem may start plummeting (Joseph, 2017). Low self-esteem is correlated with depression, eating disorders, suicidal ideation, anxiety, etc. It is also correlated with the development of OCD (Afifi, 2022).

Ghafoor and Mohsin (2013), in an effort to identify the relationship between religiosity, guilt, and self-esteem for those with OCD, conducted a study and wrote a corresponding article. They are quoted as saying, "that there is an inverse relationship between self-esteem and religiosity and OCD." This clarifies that the two do in fact have some correlation and more research needs to be done to study this relationship (Ghafoor & Mohsin, 2013).

This religious trauma among the LGBTQ+ community often leads to internalized and sometimes even outwardly expressed homophobia. Internalized homophobia presents itself when an individual is surrounded by a group's negative views, stereotypes, and stigmas regarding LGBTQ members and relationships (Gill & Randhawa, 2021). Many people in these cultures, religions, or families begin to take those ideas and turn them inward and become convinced that they must be factual. This often results in self-hatred and denial of one's true self. In fact, it was even found that there was little difference

in internalized homophobia between denominations that were very traditional and those that painted themselves as "more accepting." It was found that the less queer individuals attended these meetings, the less internalized homophobia they exhibited, and the better their mental health was. The severity and prevalence of this phenomenon for queer individuals is even stronger among those that also belong to minority groups, especially Black and Latinx individuals (Barnes, 2013).

A group of researchers led by Caitlin Pinciotti (2022) recognized that, like other minorities, those in the LGBTQ+ community are subject to minority stress because of all these different forms of homophobia. They also could see that many times OCD developed by these individuals often, "inadvertently reinforces anti-lesbian, gay, bisexual, transgender, queer/ questioning (LGBTQ+) stigma and contributed to minority stress in clients, treatment providers, and society at large" (Pinciotti et al., 2022). In their conclusion, they emphasized that a big way to help in the treatment is to "eliminate exposure to minority stress." Minority stress is experienced in incredibly high levels by queer people in the LDS church (Kehller, 2009). This can be very detrimental to the treatment of those queer individuals with OCD.

Religious trauma can also present itself in simple decisions of everyday life. Having a religion that makes so many of your decisions for you and warns you of the consequences of making the wrong ones can result in paralyzing fear. This is especially true for those in the queer community who have already been taught that they cannot trust themselves and their instincts. Nitisco (2021), a clinical psychologist and researcher, examined the phenomenon of OCD and decision making and found that, "decision-making has been proposed to have a central role in obsessive-

compulsive disorder (OCD) etiology, since patients show pathological doubt and an apparent inability to make decisions" (Nisticò et al., 2021). Combining the research of the past decade, she further explores how this symptom can be debilitating. Here we can see yet again how this can easily make the LDS church environment an easy place to foster OCD symptomatology.

The seemingly most obvious solution to this issue is for those affected to simply leave the religion or not join it in the first place. Unfortunately, it is never quite that easy. A lot of people are born into it without any choice or say in it. It then becomes all they know. Not only that, but it can serve as a foundation of their families or communities, so leaving the religion also means leaving any social support they have ever known. This is especially true of high demand religions. There are many stories of people leaving the church after attending Brigham Young University, an LDS university, and losing their job, friends, academic scholarships, important familial relationships, structured social events, etc. Often it is easier for people to intellectually understand that they don't believe in a certain religion and their teachings, especially when it comes to social issues, but when it comes to the emotional aspects of these same processes, it becomes increasingly more difficult (Winell, 2021).

A very publicized case of this regarding the queer community and the LDS religion is that of Josh

Weed. He grew up in the Mormon church and was also openly gay. Most likely due to some of the reasons mentioned earlier, he decided to stay in the religion. Not only that, but because he had also been taught that marriage in the temple is essential, he ended up marrying and having children with a straight woman. He didn't do this in the more common way where he hid his identity and tried to deny it. Both he, his wife, and everyone involved knew he was gay, and she wasn't. He became the poster child in the LDS church for how one could overcome the "sin" of homosexuality. He and his family lived this way for many years, they had four children, and claimed to be happy with their choice to live in "righteousness," but in 2018 things came to a turning point. On January 25, 2018, the seemingly happy couple declared that they were getting a divorce. Through Josh's blog, many people got to see firsthand how this deconstruction took place. Josh listed many things that had been taught to him by religious leaders, therapists, and mentors that created cognitive dissonance. He, like many others, was told that his sexual orientation was a trial, evil, not even real, something to be endured, something that could be changed in this life or the next. This kind of psychological discomfort is something that is experienced all too often, but not widely studied. This present study will work to close some of the gaps in the literature.

Although some people do leave these churches,

there is still trauma to be worked through. But the question is, just how much? One participant during the first survey made the comment while completing the questionnaire that, "The impacts of being indoctrinated from birth for 14-15 years has impacted my whole adult life" (Christensen, 2022). This begs the question of what happens when someone is in the church well into their middle age? Or how does it affect someone who leaves before they hit their teen years? It is hypothesized that the longer a queer person spends experiencing conflict between their sexuality and their church membership, the more OCD, depression, and other religious trauma symptoms they will exhibit, *unless* they successfully exit from the church.

METHODS
Participants

Participants were recruited through online advertisements (described in detail below). Inclusion criteria included (1) being 18 years of age or older (2) identifying as LGBTQ+ or same-gender attracted, and (3) identifying as a current or former member of The Church of Jesus Christ of Latter Day Saints. After excluding participants who declined to provide information on the age at which they were most concerned about being Mormon and LGBTQ+, we had a total of 1214 respondents, with a mean age of 29.9 (SD = 7.72). In all, 47.8% were assigned male at birth, 52.2% were assigned female, and 17.8 % had a gender identity different from their birth- assigned gender. Regarding church

activity, 64% reported that they remained active in the church at least once a week. Demographic information on the sample can be viewed in Table 1 (see Appendix).

Measures

For the purposes of this paper, the measures that were used were the CESD-R Depression scale (Eaton, Smith, Ybarra, Muntaner, & Tien, 2004), the Penn Inventory of Scrupulosity (PIOS; Abramowitz, Huppert, Cohen, Tolin, & Cahill, 2002), the Social Safety Scale (Diamond, 2023), the Obsessive Compulsive Inventory Scale (OCI) (Foa, Kozak, Salkovskis, Coles, & Amir, 1998), a revised version of the Secondary Traumatic Stress Scale (STSS) for the assessment of religious trauma (Bride, Robinson, Yegidis, & Figley, 2004), and self-report measures of the duration of individuals' experience of conflict between their sexual/gender identity and the church.

THE CENTER FOR EPIDEMIOLOGIC STUDIES DEPRESSION SCALE REVISED (CESD-R)

This scale is designed as a 20 question, self-report scale that aims to quantify depressive symptoms. After many tests it has been found to have strong internal consistency as well as validity and repeatability. The scale assesses common symptoms of depression such as feelings of guilt or worthlessness, changes in appetite, poor sleep quality, depressed mood, etc. The participants are asked how often they feel said items and have

options ranging from "rarely or none of the time" (0), to "most or all of the time" (4). The Cronbach's alpha for the CES total scales was 0.90 and the mean for this sample was 24.3 (*SD*=12.75). Scores between 16-23 are typically interpreted as indicating moderate depressive symptomatology whereas scores greater than 24 are interpreted as indicating severe depressive symptomatology (Radloff, 1977).

PENN INVENTORY OF SCRUPULOSITY (PIOS)

This measure is split into two subscales which are the Fear of Sin and Fear of God. It is a self-report scale containing 19 items and is used to measure religious obsessive-compulsive symptoms. Each question is responded to on a five-point scale, with frequency items ranging from 0 (never) to 5 (constantly), and distress of symptoms items rated from 0 (not at all distressing) to 4 (extremely distressing) (Abramowitz et al, 2002). Cronbach's alpha has been calculated at α=0.94 for this measure in the study. The mean was calculated at 36.8 (*SD*=11.74). *Social Safety*

The Social Safety Questionnaire asks participants to report on their social experiences within 7 different social domains: household, family, close friends, work/school colleagues, one's most important identity group, members of the LDS church, people in public spaces, and people known through social media. For each setting, participants rated on a 1-5 scale how often they (1) looked forward to seeing the people there, (2) felt certain about how things would go, (3) felt that others would notice or care if they were sick or hurt, (4) felt so comfortable

that they didn't notice time passing, (5) saw or heard something that made them feel affirmed, (6) felt that they mattered to the people there, (7) felt they could say "no" to these people, (8) felt there was someone in this setting they could turn to for help, (9) felt so secure that they stopped paying attention to how others perceived them, (10) felt that they were treated and spoken to the way they wanted, (11) were made to laugh or feel good by others, (12) felt like their real self, and (13) experienced joy and pleasure. Cronbach's alpha ranged from .84 to .95 for each separate domain, and reliability for the total social safety score (averaged across all domains) was .95. Mean social safety was 3.09 (SD = .47).

SECONDARY TRAUMATIC STRESS SCALE (STSS)

The STSS is made up of 17-item self-report measures. It is designed to measure PTSD- related symptoms of intrusion, avoidance, and arousal. The questions are scored on a five-point Likert-like scale, each ranging from "never" (1) to "very often" (5), indicating how often symptoms were felt in the last week. Items were summed for this study. Though the symptoms measured are generally associated with secondary traumatic stress, Simmons (2017) determined that this would provide enough sensitivity to measure the impacts of both unintentional and intentional traumatic experiences, only modifying it to refer better to one's religious experiences and finding high internal reliability for his sample. This modified version was the one used in the present survey. We calculated Cronbach's alpha to be α=0.81 for

this measure. The mean was calculated at 59.33 (*SD*=5.51).

OBSESSIVE COMPULSIVE INVENTORY SCALE

The OCI, Obsessive Compulsive Inventory, Scale is a self-report measure used to assess the severity of OCD symptoms. The OCI scale consists of 18 items that assess various aspects of OCD symptoms, including checking, washing, obsessing, hoarding, and ordering. Each item is rated on a five-point Likert scale, with responses ranging from 0 (not at all) to 4 (extremely). The total score on the OCI scale ranges from 0 to 72, with higher scores indicating greater severity of OCD symptoms. The OCI scale is a commonly used and well-validated measure for assessing OCD symptoms in clinical and research settings. Cronbach's alpha has been calculated at α=0.96 for this measure in the study. The mean was calculated at 44.0 (*SD*=22.79). A score of 28 is typically interpreted as indicating the presence of OCD.

Duration of Concern

The questionnaire asked individuals to report the age at which they felt the most concern about being LGBTQ+ and Mormon. The mean age of greatest concern was 17.88 (*SD* = 5.1). We subtracted this age from participants' current age to estimate the length of time they experienced concerns about being LGBTQ+ and Mormon. The mean duration of concern was 12.0 years (*SD* = 7.5).

Procedure

Participants who encountered online advertisements of the survey were instructed to contact the investigators via email or to visit the study website (www.matteringmatters.net) which provided more information about the study and also included a direct link to participate. The survey began with the IRB-approved consent form, and participants indicated their consent by clicking at the bottom of the form to progress to the survey. Participants were sent a $40 gift card after completing the survey. Six percent of participants chose to donate their gift card to other participants.

Analytic Strategy

All analyses were conducted with multivariate regression analysis in SPSS. All continuous predictor variables were centered before entry into the regression model, and all dichotomous variables were dummy coded.

RESULTS

For the multivariate regression analysis, the outcome variables were depressive symptoms, scrupulosity, religious trauma symptoms, obsessive-compulsive symptoms, and social safety. The predictors were participants' current age, their birth-assigned gender, the duration of their concerns with being LGBTQ+ and Mormon, and whether they currently remained active in the church. The results indicated that individuals who had spent more years feeling concerned about

being LGBTQ+ and Mormon reported significantly higher levels of depression (b =.45, p < .001), higher obsessive-compulsive symptoms (b = .45, p < .001), and lower social safety (b = −.01, p = .006), but there was no association between duration of concern and participant's religious trauma symptoms (b= -.01, p = .10) or their scrupulosity (b = .01, p = .62). We then conducted exploratory tests for interactions between duration of concern and the other variables in the model (age, gender, and current membership status). We found significant interactions between participant's age and participant's duration of concern for scrupulosity (b = -.01, p < .001), social safety (b = .01, p < .001), and obsessive-compulsive symptoms (b = -.023, p < .001), and we found significant interactions between participant's current church activity and their duration of concern for depression (b = .376, p < .001) and social safety (b = -.012, p <.001). These interactions can be seen in Figures 1-5 (see Appendix).

Follow-up simple slope tests (estimated at one standard deviation above and below the sample mean for age) revealed that among younger participants (those in their early 20's), participants' duration of concern was significantly associated with higher scrupulosity, b = .02, p< .001, whereas duration of concern was not significantly associated with higher scrupulosity among older participants (those in their late 30's or older; b = .01, p = .10). For social safety, simple slopes tests revealed that duration of concern was more strongly associated with lower levels of social safety among younger than older

participants (*byounger* = -.03, *p* < .001, *bolder* =-.01, *p* < .001). This can be seen in Figures 1 and 2 (see Appendix).

Similarly, for obsessive-compulsive symptoms, duration of concern was more positively associated with obsessive-compulsive symptoms among younger than older participants (*byounger*= .80, *p* < .001, *bolder* =.42, *p* < .001). This can be seen in Figure 3. (see Appendix).

With respect to church activity, duration of concern was more strongly positively associated with depressive symptoms among individuals still active in the church (*b* = .71, *p* <.001) than among individuals no longer active (*b* = .20, *p* = .03), and duration of concern was negatively associated with social safety only among active church members *(bactive* = -.02, *p* <.001, *binactive* = -.001, *p* .43). This can be seen in Figures 4 and 5 (see Appendix).

DISCUSSION

The purpose of this research was to study the impact of religious trauma (specifically, OCD symptoms related to the LDS faith) and social safety on the mental health complications that many individuals who identify as part of the LGBTQ+ community and belong to the LDS faith report. The present study specifically aimed to explore the associations between duration of time spent in the LDS faith and the severity of OCD and other mental health symptoms.

The hypothesis proposed was that the longer duration a queer individual had in the LDS church, the more scrupulosity OCD symptoms they would report. The findings align with both this hypothesis and prior literature on the subject as discussed in the introduction; however, the correlation was only found in younger participants. This may suggest that those younger participants with a longer duration may require a different type of intervention than those with a short

duration, or those with a longer duration but who are older. This could be due to the importance of developmental years on an individual's brain and sense of self (Goldback & Gibbs, 2017).

The second hypothesis suggested that the longer the duration a queer individual had in the LDS church, the more depressive symptoms would be present. The findings supported this hypothesis as well. It also makes sense when compared to prior literature which suggests that "stress from hiding and managing a socially stigmatized identity" can be a risk factor for depression (Hall, 2018). This idea is related to that of social safety as well. The findings also supported the idea that less social safety would be reported the longer the individual spent in the church.

Altogether, the results of this study suggested that individuals who encountered more religious beliefs and teachings for an extended period of time experienced higher levels of

religious trauma. Additionally, exposure to harmful religious teachings/beliefs was a significant predictor of scrupulosity OCD symptoms, depression, lack of social safety and overall worse mental health outcomes. It is likely that the harmful effects of these religious teachings on people's health is a direct result of the trauma that these teachings can often induce.

This study's results are consistent with previous research, including research released by Simmons in 2017 that found a connection between exposure to harmful beliefs/teachings in the LDS religion and religious trauma. The study conducted for this

paper went even further, discovering that religious trauma is correlated with worse mental health, thus connecting the two areas of literature.

Many of these findings were the same as were found in the previous study (Christensen, 2022). However, this extension of the study also found a high correlation between duration spent in the church with the severity of religious trauma symptomatology. This implies that there may be a need for specific interventions designed for individuals who have been in the LDS church and experienced religious trauma for an extended period of time.

Limitations and Future Directions

While this study has many strengths, there are a few limitations that are important to note. One limitation is the use of convenience sampling, which may limit the generalizability of the results to the population of interest (LGBTQ+ individuals with past or present membership within the LDS church). Another limitation is the fact that the study is essentially retrospective, as respondents were asked to consider the impact of past events. This may affect the accuracy of responses as people's memories are not always 100% accurate (Gloster et al., 2008). Future studies should be mindful of this and potentially have a way to report during this experience instead of after.

The lack of a control group that is made up of heterosexual and gender conforming LDS members

also limits the ability to determine the full impact of LDS participation on LGBTQ+ members. Future studies should include a control group to increase the validity of results. A third limitation is the fact that the survey was long enough (60-120 minutes) that some participants didn't finish, so the data we got was just based on people that had the time and resources to commit to starting and finishing the survey. Those who do future research on this topic may want to consider that factor when they decide on a form of data collection.

Despite these limitations, the study found that more time spent within the LDS church predicted worse mental health outcomes, and having spent less time exposed to this is associated with better mental health outcomes. An alternative explanation may be that people with greater mental health might have felt more empowered to leave sooner; however, more research is needed to fully analyze this possibility.

These findings have important implications, suggesting the need for greater support for members of the LDS church who identify as LGBTQ+ individuals, especially those who are still active in the church and those who have or are spending an extended period of their time in life participating in the church. Further research is necessary to explore and test these implications.

Conclusion

Despite the limitations mentioned above, the

study yielded significant results that have important implications for many LDS and queer communities. One of the biggest ones is that many LGBTQ+ individuals in the LDS church have been exposed to harmful teachings and beliefs, which have led to trauma that is associated with negative mental health outcomes such as depression, scrupulosity, and poor general health.

The study also found that having spent a longer amount of time in the LDS church intensifies the severity of symptoms experienced, including scrupulosity OCD, depression, and other health outcomes. This may imply that those having spent more time may require a different or more intensive approach to treatment. Further research is required to validate and expand upon these implications.

REFERENCES

Afifi, D. Y., Shahin, M. O., Alaa, Y., & Ayoub, D. R. (2022). Investigation of Symptom Severity, Self-Esteem, & Suicidality in Anxiety Disorders and Obsessive Compulsive Disorder. *Revista Iberoamericana de Psicología Del Ejercicio y El Deporte*, *17*(6), 380–386.

Albińska, P. (2022). Scrupulosity — cognitive-behavioural understanding of religious/moral obsessive-compulsive disorder. *Psychiatria i Psychologia Kliniczna (Journal of Psychiatry & Clinical Psychology)*, *22*(1), 25–39. https://doi-org.ezproxy.lib.utah.edu/10.15557/PiPK.2022.0004

Allen, G. E. K., Wang, K. T., & Stokes, H. (2015). Examining legalism, scrupulosity, family perfectionism, and psychological adjustment among LDS individuals. *Mental Health, Religion &*

Culture, *18*(4), 246–258. https://doi- org.ezproxy.lib.utah.edu/
10.1080/13674676.2015.1021312

Ashwortth, K. (2022, February 5). *In My Own Words – Latter
Gay Stories Podcast.* Latter Gay Stories Podcast.
https://lattergaystories.org/inmyownwords/

Avance, R. (2013). Seeing the light: Mormon conversion and
deconversion narratives in off- and online worlds. Journal of
Media and Religion, 12(1), 16-24. doi:10.1080/
15348423.2013.76038

Barnes, D. M., & Meyer, I. H. (2012). Religious affiliation,
internalized homophobia, and mental health in lesbians, gay
men, and bisexuals. *The American journal of orthopsychiatry*,
82(4), 505–515. https://doi.org/10.1111/j.1939-
0025.2012.01185.

Brandley, B. (2020). "This is How You Navigate the World":
Impacts of Mormon Rhetoric on White Queer Members'
Identity Performances. *University of New Mexico Digital
Repository.*

Foa, E. B., Kozak, M. J., Salkovskis, P. M., Coles, M. E., &
Amir, N. (1998). The validation of a new obsessive–compulsive
disorder scale: The Obsessive– Compulsive Inventory.
Psychological Assessment, 10, 206-214. https://doi.org/10.1037/
1040-3590.10.3.206

Gill, S., & Randhawa, A. (2021). Internalised Homophobia
and Mental Health. *Indian Journal of Health & Wellbeing*, *12*(4),
501–504.

Gloster, A. T., Richard, D. C. S., Himle, J., Koch, E., Anson, H.,
Lokers, L., & Thornton,

J. (2008). Accuracy of retrospective memory and covariation
estimation in patients with obsessive–compulsive disorder.
Behaviour Research & Therapy, *46*(5), 642–655. https://doi-
org.ezproxy.lib.utah.edu/10.1016/j.brat.2008.02.010

Goldbach, J. T., & Gibbs, J. J. (2017). A developmentally informed adaptation of minority stress for sexual minority adolescents. *Journal of adolescence*, *55*, 36–

50. https://doi.org/10.1016/j.adolescence.2016.12.007

Hall, W. J. (2018). Psychosocial Risk and Protective Factors for Depression Among Lesbian, Gay, Bisexual, and Queer Youth: A Systematic Review. *Journal of Homosexuality*, *65*(3), 263–316. https://doi-   org.ezproxy.lib.utah.edu/10.1080/00918369.2017.1317467

Hollier, J., Clifton, S., & Smith-Merry, J. (2022). Mechanisms of religious trauma amongst queer people in Australia's evangelical churches. *Clinical Social Work Journal*, *50*(3), 275–285. https://doi-org.ezproxy.lib.utah.edu/10.1007/s10615-022-00839-x

Joseph, L. J., & Cranney, S. (2017). Self-esteem among lesbian, gay, bisexual and same- sex-attracted Mormons and ex-Mormons. *Mental Health, Religion & Culture*,

20(10), 1028–1041. https://doi-   org.ezproxy.lib.utah.edu/10.1080/13674676.2018.1435634

Kelleher, C. (2009). Minority stress and health: Implications for lesbian, gay, bisexual, transgender, and questioning (LGBTQ) young people. *Counselling Psychology Quarterly*, *22*(4), 373–379. https://doi.org/10.1080/09515070903334995

Kimball, S. L. (1969). *The Miracle of Forgiveness*. Bookcraft, Salt Lake City. Kingdon. (n.d.). *Religious Trauma & Transitions — RESTORATION COUNSELING.*

RESTORATION COUNSELING.

https://www.restorationcounselingseattle.com/religious-trauma-

transitions#:~:text=Religious%20Trauma%20Syndrome%20(RTS

)%20is,traumati

c%20or%20stressful%20religious%20experiences.

McIngvale, E., Rufino, K., Ehlers, M., & Hart, J. (2017). An In-Depth Look at the Scrupulosity Dimension of Obsessive-Compulsive Disorder. *Journal of Spirituality in Mental Health*, *19*(4), 295–305. https://doi- org.ezproxy.lib.utah.edu/10.1080/ 19349637.2017.1288075

Myers, Summer Anne, "Visualizing the Transition Out of High-Demand Religions" (2017). LMU/LLS Theses and Dissertations. 321. https://digitalcommons.lmu.edu/etd/321

Page, M. J. L., Lindahl, K. M., & Malik, N. M. (2013). The Role of Religion and Stress in Sexual Identity and Mental Health Among Lesbian, Gay, and Bisexual Youth. *Journal of Research on Adolescence (Wiley-Blackwell)*, *23*(4), 665–677. https://doi.org/10.1111/jora.12025

Radloff, L. S. (1977). The CES-D Scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement, 1*(3), 385-401

Village, A., & Francis, L. J. (2008). Attitude Toward Homosexuality among Anglicans in England: the Effects of Theological Orientation and Personality. *Journal of Empirical Theology*, *21*(1), 68–87. https://doi- org.ezproxy.lib.utah.edu/ 10.1163/092229308X310740

Zhang, Y., Qu, B., Lun, S., Guo, Y., & Liu, J. (2012). The 36-Item Short Form Health Survey: Reliability and Validity in Chinese Medical Students. *International Journal of Medical Sciences*, *9*(7), 521–526. https://doi.org/10.7150/ijms.4503

APPENDIX

Figure 1

Interaction duration of period of greatest concern (PGC) and age on social safety
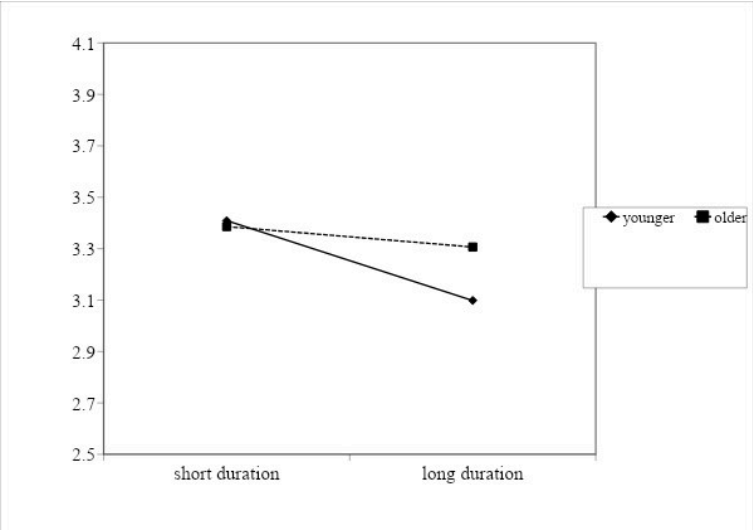
Figure 2

Interaction between duration of period of greatest concern (PGC) and age on scrupulosity
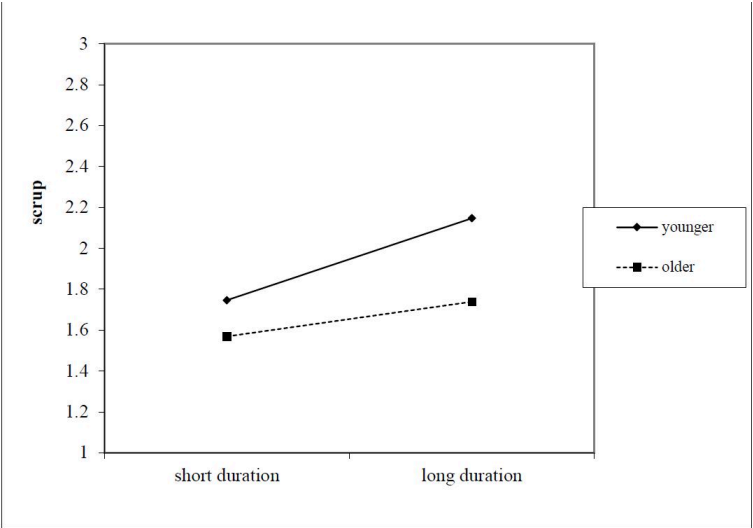
Figure 3

Interaction between duration of period of greatest concern (PGC) and age on OCD
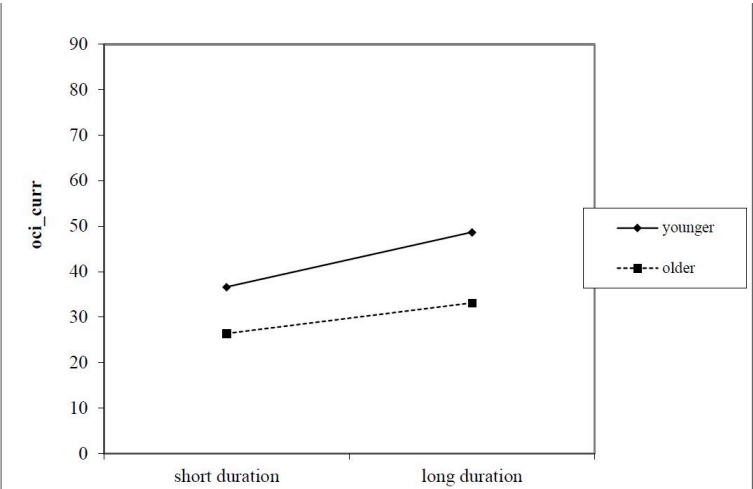
Figure 4

Interaction between duration of period of greatest concern (PGC) and current church activity on depression
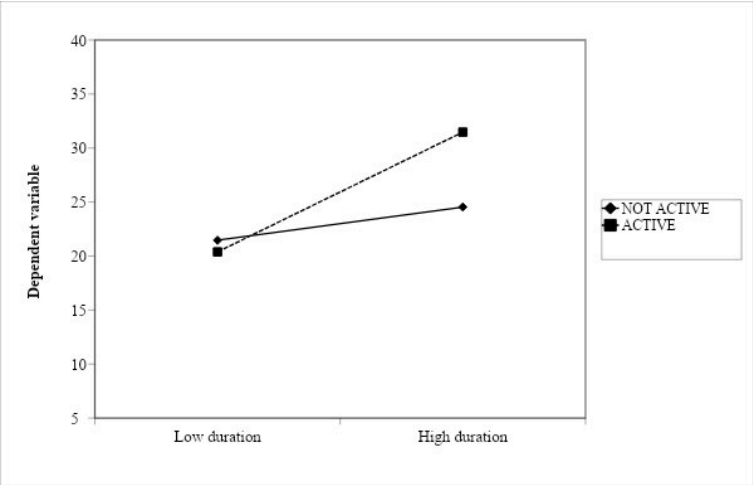
Figure 5

Interaction between duration of period of greatest concern (PGC) and current church activity on social safety
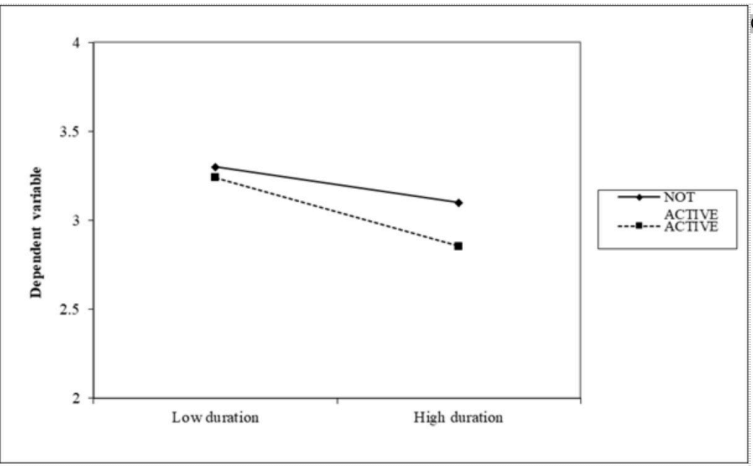
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130. **Sustainable Support for America's Children: The Expanded Child Tax Credit of 2021 and the Pressing Need to Implement a Bipartisan, Long-Term Child Allowance Policy in the United States**

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ABSTRACT

The United States has a vested interest in providing support for families raising children. Over the past 25 years, the country has used a tax code provision known as the Child Tax Credit (CTC) to provide financial assistance to parents raising children. This paper presents a synthesis of relevant political and academic discourse on child tax credits and allowances over the past 25 years and offer a sampling of potential paths forward for the policy in the United States. The research affirms that the United States should focus its efforts on converting to a fully refundable, simple-to-navigate, and monthly federal child allowance to maximize its return on investment for American families.

Academic understanding of child poverty's detrimental and long-term effects is well documented. Research links growing up poor to low academic achievement, school dropout, abuse and neglect, and developmental and behavioral issues (Nikulina et al., 2011). Children who live in poverty are more likely as adults to develop and die earlier from cardiovascular disease and type II diabetes. Studies show that adverse health impacts from these early social determinants are biologically embedded, meaning that improved

circumstances in adulthood only moderately correct the issues (Raphael, 2011). In 2008, economists estimated that child poverty costs the United States $500 billion a year, reduces productivity and economic output by 1.3 percent of GDP, raises crime, and increases health expenditure (Holzer et al., 2008).

The welfare system should be structured in a way that supports, not misses, those that are dedicated workers, devoted parents, and valuable contributors to their communities. Unfortunately, actually obtaining government benefits can be like a full- time job for many people, which creates the opposite effect of what a means-tested social welfare system is supposed to accomplish — providing a safety net while encouraging work and strong families.

Reforming or replacing the Child Tax Credit is a great place to start. Children who grow up in poverty face poorer educational, health, and career outcomes than their middle-class and wealthy peers regardless of their innate curiosity, intelligence, and drive. There is bipartisan momentum behind expansion and simplification of the Child Tax Credit, including proposals that include transforming the tax credit into a monthly child allowance.

The United States should convert the Child Tax Credit into a child allowance policy because such a change would provide more sufficient financial

support and more consistent and predictable income for families and children, and it would be more easily and effectively administered by federal agencies. An intelligent and informed replacement of the Child Tax Credit with a child allowance policy can boost the well- being of American families, particularly those that face the greatest barriers to the American Dream. The prospect of raising a child will become less daunting, and parents will be incentivized and empowered to contribute to society both at work and at home. Children will be better equipped to thrive and reach their potential. A well-designed and administered benefit should stimulate both economic growth and citizen happiness.

First, this paper analyzes the history of the Child Tax Credit in the United States with a specific focus on the short-lived Expanded CTC that came in response to the coronavirus pandemic. Next, this paper will attempt to analyze the socioeconomic effects of child allowance policy choices, discussing the scale of need for and use of funds by parents as well as impacts on fertility rates. Afterwards, the paper will explore possible solutions by analyzing child allowance programs in wealthy countries around the world, using historical and contemporary political discourse to analyze ideological perspectives and approaches to the CTC and child and family policy, and breaking down

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currently pending CTC proposals among thought leaders and legislators in the United States.

Finally, it will conclude with the author's perspective on three key principles to guide the design and implementation of a permanent federal child allowance program in the United States.

HISTORY OF THE CHILD TAX CREDIT

The Child Tax Credit: 1997-2020

The Taxpayer Relief Act of 1997, passed by a Republican-controlled Congress with bipartisan support and signed into law by President Bill Clinton, reduced many federal taxes and introduced several new tax credits (Crandall-Hollick, 2021). Notable provisions included an 8% reduction in the top marginal long-term capital gains rate, the establishment of Roth IRAs as retirement accounts exempt from capital gains taxes, and the introduction of the Child Tax Credit.

The Child Tax Credit initially provided a $400 benefit for each child under age 17 but was increased to $500 in 1998. The credit was applied annually as part of an individual's tax return. The credit was nonrefundable, only decreasing existing federal tax liability. If an individual did not owe any more taxes, the government did not return the leftover credit as a cash benefit. The credit phased out for taxpayers with incomes over $110,000 (married filing jointly) or $75,000 (head of household or single).

The Economic Growth and Tax Relief

Reconciliation Act of 2001 increased the credit to $600 per child and scheduled stepped increases to $1,000 per child by 2010 (subsequent legislation accelerated these increases and reached $1,000 per child in 2004). The legislation also made the credit semi-refundable: lower-income taxpayers with more than $10,000 of earned income could receive part or all of the child tax credit as a cash benefit. Known as the "refundability threshold," the $10,000 limit was adjusted annually for inflation. In 2009, President Barack Obama signed the American Recovery and Reinvestment Act in response to the financial crisis. This act temporarily expanded the refundability of the Child Tax Credit by reducing the refundability threshold to $3,000 for 2009 and 2010. This change was made permanent through later acts of Congress.

In 2017, a Republican-dominated Congress passed The Tax Cuts and Jobs Act, most notable for its sweeping reductions in federal taxes, which included a variety of modifications to the Child Tax Credit. The act increased the maximum benefit to $2,000 and decreased the refundability threshold to $2,500. The phase-out threshold was raised significantly to $400,000 for married joint filers and $200,000 for head of household or single filers. By making the single filer threshold exactly half of the married threshold, Republican lawmakers intended to address a tax disincentive for marriage, a longstanding conservative priority in tax reform.

The act required that taxpayers include a work-authorized Social Security Number when filing for the credit. Congress made the provision with the intent of limiting fraudulent claims, but in effect it may also make the credit less accessible to low-income families. Finally, the legislation created a temporary

$500 per dependent nonrefundable credit for children not eligible for the child tax credit.

Lessons Learned

The legislative history of the Child Tax Credit shows lawmakers continually coming back to a few key questions. How large of a benefit should the CTC be? Should the credit be refundable, and if so, should it be fully or partially refundable? Should filers be required to earn income in order to qualify for the CTC, and if so, how much should they make? At what income level should the credit phase out? Answers to these questions generate the foundational structure of the child tax credit, which creates important impacts on children's lives.

Throughout the Child Tax Credit's legislative history, initially temporary modifications to increase the scope and size of the credit are generally extended and made permanent in subsequent years as the Child Tax Credit proves to be successful, effective, and popular. Figure 1 below from the Congressional Research Service shows the evolution of the Child Tax Credit over time with respect to a married couple with one child under 5 years old. The Y-axis shows the credit amount received and the X-axis shows household income, and the various lines show the policies enacted over the period.

Figure 1

Child Credit Amount by Income for Selected Legislation, 1997-2021

Married Couple with One Child (0-5 years old)

Credit amount ($)

Note. From Crandall-Hollick, M. L. (2021). The Child Tax Credit: Legislative History.

Congressional Research Service, R45124. https://sgp.fas.org/crs/misc/R45124.pdf

The Expanded Child Tax Credit of 2021

The COVID-19 pandemic exacerbated already significant problems for low- income parents raising children. Widespread school closures created high social and economic costs, including stymied academic progress, lack of access to childcare and nutritious food, and social isolation.7 In response, the American Rescue Plan Act of 2021 created the expanded Child Tax Credit. Simply calling it the expanded Child Tax Credit is misleading, however, as the

existing tax credit program was converted into a child allowance program.

The government increased the Child Tax Credit from $2,000 per child to $3,000 per child for children over the age of six and $3,600 for children under the age of six. The credit was paid in monthly increments of $250 or $300 rather than an annual lump sum. It was fully refundable, meaning that families at low income levels that do not have outstanding tax liability would receive the credit as a direct cash transfer. The expansion raised the qualifying age limit from 16 to 17 and adjusted the income phase-out threshold down to $150,000 for joint filers and $112,500 for single or head of household filers.

After that income level, the credit returned to the previous level as established in 2017 by the Tax Cuts and Jobs Act. The top line in Figure 1, in pink, is the American Rescue Plan Act. Note that at an income level of $0, a family qualified for the benefit, a first for any version of the legislation.

At the time of this writing, Congress has been unable to find a compromise to renew the CTC expansion for 2022 and beyond. The CTC has reverted to the previous iteration: only $2,000 per child without full refundability and revoked qualification for 17-year-olds. In the absence of the monthly cash payments guaranteed by the expanded credit, about 3.7 million more American children are living in poverty, according to the Center on Poverty and Social Policy, at Columbia University (Parolin et al., 2022).

CHILD ALLOWANCE POLICY OVERVIEW
Socioeconomic and Health Impacts

In 2020, researchers conducted a longitudinal study on the effects of the Child Tax Credit with

a nationally representative sample of mothers and their biological children. Some of the most significant findings included that children suffered fewer injuries requiring medical attention and dealt with fewer behavior problems due to CTC payments. Alleviating financial strain through the policy also decreased child abuse and youth violence. However, the researchers found that these effects were present "only when it was partially refundable (i.e., mothers could receive a tax refund for a portion of the CTC that exceeds their tax liability) for families making as little as $3000 a year" (Rostad et al., 2020). These findings highlight the critical nature of refundability for the most vulnerable parents and children. The authors concluded, "Tax credits like the CTC have the potential to alleviate financial strain among families, and consequently, may have impacts on injury and behavior problems." While direct financial support cannot cure all social ills for low-income families, this study provides evidence that alleviating financial strain can lead to positive impacts on health and overall wellbeing.

Earlier this year, a major study found that a predictable, monthly unconditional cash transfer given to low-income families may have a causal relationship with improved infant brain activity. By creating a more auspicious financial situation for their families, children's brain activity adapted in

ways associated with the development of critical cognitive skills (Troller-Renfree et al., 2022).

In 2007, Mayor Mike Bloomberg implemented Opportunity NYC, a program giving cash to low-income families in New York City meeting specific requirements, including regular school attendance and doctor visits. The city transferred an average of $8,700 to each participating family over three years, and the results were encouraging. Families faced reduced food scarcity and financial worries while increasing their savings and health insurance. Still, there were no significant positive increases in educational outcomes. NYU professor Lawrence Aber explained this underwhelming result by asserting that the program should have been longer-lasting and rewarded more measures of academic improvement. Overall, however, the program's results were impressive, and more experiments of a similar nature could generate a better understanding of best practices and generate momentum for a long-term policy solution (Chang & Madrick, 2016). At the very least, the program should pique policymaker interest to continue to experiment with cash transfer welfare programs at local levels.

Impacts on the Economy

The Niskanen Center, a think tank, estimated that the CTC expansion would boost consumer spending by $27 billion in rural areas, generating $1.9 billion in revenues from state and local sales

taxes (Hammond, 2021). In a report, they wrote "the CTC expansion provides larger benefits to states with lower average incomes and larger average family sizes, helping support access to community-based child care." The benefits particularly extend to rural communities, who receive a "substantial injection of relative purchasing power equivalent to 1.35% of non-metro GDP" from an expanded CTC. These result in a "multiplier effect," meaning that a dollar injected into the program results in more than one dollar of economic benefit as that dollar circulates through the economy.

Policymakers must consider these benefits when deciding the size of child allowance benefits. However, as we have seen in recent months, too much government stimulus can contribute to inflationary pressures. Leaders must take an empirical and level-headed approach to balancing these competing considerations.

Economists at Columbia University argued that permanently converting the Child Tax Credit into the expanded Child Tax Credit would cost roughly $100 billion but generate about $800 billion in benefits to society. Specific significant factors in this estimation include increased future earnings for children, decreased involvement with child protection and criminal justice services, and improved children's and parents' health outcomes (Garfinkel et al., 2021).

Estimating Need

The cost of raising a child must be understood to determine the proper size of the credit. Debates on the Child Tax Credit have primarily moved from a question of kind (should the United States provide benefits to families?) to a question of degree (how large should the benefit be, and who should qualify?). Having a Child Tax Credit is popular among legislators and voters on both sides of the aisle, and there exists no serious movement to abolish the credit among lawmakers. Both Republicans and Democrats have led efforts to expand the credit in the past. Ultimately, Congress must decide how much and for whom the government should bear the cost of raising children.

Researchers can use various methods to attempt to measure the cost of raising a child. Martin Browning of McMaster University highlighted that necessary considerations include how children affect the expenditure patterns of a household, how much income a family with a child needs compared to a childless family, and how much parents actually spend on their children (Browning, 1992). A few notable estimates are included below as a glimpse of studies of the cost of raising children.

The U.S. Department of Agriculture has provided estimates of expenditures on children from birth through age 17 since 1960. Their method includes allocating child- specific expenses directly to children while using findings from Federal surveys

on children's budget shares to analyze children's contributions to other costs. In 2015, the USDA estimated that it costs an average of $233,610 for a middle-class household to raise a child to age 18, or about $13,000 per year. For those making under $59,200 annually, annual expenditures ranged between $9,330 and $9,980, while those making above $107,400 spent between $19,380 to $23,380 per year per child. The study also notes that 'economies of scale' exist as more children are introduced to the family — sharing rooms and passing down items like clothes, toys, and books makes each successive child less expensive to raise than the one before (Lino, 2015).

In Christopher A. Sarlo's paper entitled *The Cost of Raising a Child,* he argues that "prevailing cost estimates have a distinct middle-class bias and do not reflect the reality of raising children in lower income and newer immigrant households. There is a concern that such estimates send a clear message to lower income families that they really cannot afford children and, perhaps, shouldn't have any." His report concludes that an annual payment $3,000 to $4,500 would be sufficient for the bare necessities and basic costs necessary for a child's healthy development (C. Sarlo, 2013).

A policy that provides more support to younger children than to older children is logical, as parents tend to have lower incomes when they first have children but will hopefully build savings over time.

Structuring the policy in this way may also reduce barriers to younger people becoming parents.

Analyzing Use

Some decision-makers worry about the implications of providing any income support to low-income individuals without strings attached to the use of the money. Many think non-disabled individuals will use the extra influx of cash to avoid work, deepening their dependence on government aid. Senator Joe Manchin reportedly expressed concerns to Congressional colleagues that parents would use their expanded child tax credit payments to buy drugs (Shabad et al., 2021). It is worth analyzing the extent to which these concerns are valid.

The Brookings Institute utilized a probability-based online panel to survey a nationally representative group of 1,514 U.S. parents eligible for the credit, administered immediately before the they received their first CTC payments (Jabbari et al., 2021). As part of this study, participants were asked how they planned to use their CTC payments. Top responses included using the funds to build emergency savings (75%), paying routine expenses (67%), purchasing essential items for children (58%), purchasing more or better food (49%), starting or growing a college fund (42%), and paying for child activities (42%). Parents also noted that they would use the money for moving or making home improvements (32%), health care

(29%), and child care (26%) expenses. Other uses included spending more time with children (20%), purchasing gifts or entertainment (20%), paying for tutors (7%), working less or changing jobs (6%), or sending their children to a different school (6%).

Comparing between income groups, a substantially more significant proportion of lower-income families planned on using their CTC for essential items, more and better food, and spending more time with their children than middle- and higher-income families. Brookings argued that these findings show that fears that the CTC will disincentivize work are "relatively unfounded," given that only 6% of respondents planned on using the money to work less. Instead, they argued, the expanded CTC would alleviate poverty, increase social mobility, and promote financial well-being for both parents and their children.

Effects on Labor

Early results from studies of the expanded Child Tax Credit are promising. CTC payments have helped millions of parents and caregivers enter or stay in the workforce.[20]

A study by researchers at Columbia University found that real-world analysis of labor supply shows that the CTC did not have major negative employment effects that offset its documented reductions in poverty in 2021 (Ananat et al., 2021).

The Heritage Foundation, a conservative think tank, speaking of proposed Build Back Better social spending generally, raised these objections to the policy: "It overturns the foundations of the Clinton-era welfare reform, which

required able-bodied recipients to work or prepare for work in exchange for cash benefits. Instead, the bill resurrects the long-failed policy of paying families not to work" (Rector & Hall, 2021). However, there is a growing consensus among researchers that cash transfers made without an explicit employment requirement tend to result in little to no change in labor participation, except for reductions in working hours for the elderly and some refugee groups (Baird et al., 2018).

Effects on Fertility Rates

The fertility rate of the United States reached a historic low in 2020 at 1.6 births per woman, well below the replacement level of roughly 2.1 births per woman (*Fertility Rate, Total (Births per Woman) – United States*, 2022). The rate had remained slightly above replacement level throughout most of the 1990s and 2000s before starting a gradual decline in 2007. Even before the outbreak of the coronavirus pandemic, bearing and raising children was made prohibitively costly for many prospective parents due to student loan debt, inflation, and wage stagnation. A low birthrate creates severe demographic challenges as it tasks a smaller group of young people with the burden of providing for and taking care of a growing group of aging people (Morgan, 2003).

The Archbridge Institute and the Harris Poll surveyed a representative sample of US adults on their plans for having children. The poll found that 55% of US adults under the age of 30 want to have a child, either biologically or through adoption, in the future. Among those who do not want to have a child, 46% cited their personal financial situation as influencing their decisions (Skiera, 2022). Unmet fertility occurs when the actual

number of bearing children is greater or less than the number of children than a woman desires to have because society it too difficult or expensive. Research shows that direct child allowance payments lead to at least a slight increase in birthrates.

A landscape review of studies found a positive and significant price effect on overall fertility, with benefit elasticities around 1-2% (González & Trommlerová, 2021). For example, a child allowance in Spain led to a 3 percent increase in birthrates, and birthrates dropped 6 percent when it was canceled. Further, when the government introduced the benefit, abortions dropped by .15 daily abortions per 100,000 women, and increased by .37 daily abortions per 100,000 women when the benefit was removed.

Researchers at Columbia University found that financial incentives play a notable role in determining fertility decisions in France, with the evidence suggesting that an unconditional child benefit with a direct cost of 0.3% of GDP would raise total fertility by between 15.5% and 17.7% (Laroque & Salanié, 2005).

FINDING A SOLUTION

The Ideal World

Conservative and progressive values find an intersection in a child allowance policy. Many progressives see caring for low-income parents as a necessary function of the government, while many conservatives advocate for family values and decry what they frame as the dissolution of the family unit. Some prominent Republicans, including Senator Mitt Romney (R-UT), have called attention to the United States' declining birthrate as a

precursor to socioeconomic difficulty and declining influence on the world stage (Romboy, 2021).

A recent study into the conservative and liberal views of the ideal world generated insights that can help to dissect these ideological intricacies as they relate to the Child Tax Credit and family policy more generally. Researchers analyzed over 3.8 million tweets sent by more than 1 million Twitter users about the factors that constitute a good or bad society (Sterling et al., 2019). Liberals spoke significantly more often of social justice, global inequality, women's rights, racism, criminal justice, health care, poverty, progress, social change, personal growth, and environmental sustainability. Conservatives referred to religious tradition, social order, controlled immigration, capitalism, and national symbols more often. The study also found common ground: liberals, moderates, and conservatives were equally likely to see economic prosperity, family, community, and the pursuit of health, happiness, and freedom as core pillars of the ideal society.

The three main conservative concerns about an expanded child allowance program are the burden on the already substantial federal deficit, the promotion of unstable families and single-parent households, and further reliance by low-income Americans on the welfare state.

Liberals and progressives tend to support increased help for the most vulnerable and needy in society. Equity and equality are fundamental values. The refundability portion of the expanded

Child Tax Credit is a major appeal to these values —
providing unconditional support for parents even if
they do not receive a traditional income.

The Child Tax Credit can be designed and
messaged so that the fundamental shared values
of liberals and conservatives are reflected and
emphasized. Some conservatives have concerns
about a deteriorating social fabric due to changing
gender and family roles and conduct expectations.
The CTC can promote supportive, nurturing
environments for children, resulting in increased
stability in the home and, consequently, decreased
risk for gang participation or involvement in the
criminal justice system down the line. Noting the
decrease in abortion rates other countries that have
implemented such policies may resonate for some
pro-life   conservatives.   Understanding   and
communicating the economic return on investment
for the country from such a policy can also appeal
to more capitalist-oriented conservatives who
primarily value strong economic growth and
promoting business interests.

Some aspects of the CTC should appeal to
individuals all along the spectrum. Effective child
allowance policy promotes better health and
nutrition   outcomes   and   increases   economic
mobility.   However,   decisionmakers   must
acknowledge and address a fundamental difference
between the liberal and conservative views of the
primary purpose of the child tax credit. Liberals

see the child tax credit primarily as an anti-child poverty tool. Meanwhile, conservatives see the credit as a way to promote child-bearing and family values through primarily supporting working, middle-income families (Gleckman, 2022). The policy can do both, though trade-offs are inevitable. Finding a compromise, perhaps in some form of watered-down income or work requirement, or in tweaking phase-in and phase-out levels, will be difficult but not impossible. The values presented above of conservative and liberal views on the ideal world can serve as a starting point for political leaders and elected officials to use in designing and promoting a more effective expanded and reformed CTC that can get through Congress and be signed into law.

Child Allowances Around the Globe

Many wealthy nations employ a universal child benefit. Luxembourg provides the most generous child benefit at nearly $4,500 per year for two kids. Belgium, Austria, Germany, and Ireland all provide substantial benefits ranging from $2,000 to $3,000 per year per child. Policies vary based on priorities and the extent of other social programs.

France's program, for example, is designed primarily to encourage higher birth rates — their child benefit is only available for families with two or more children (Matthews, 2016). The United Kingdom had a universal child benefit policy from 1999 until 2013. Now, Britons qualify for a Child

Benefit of £21.80 per week for an eldest or only child and £14.45 per week for each additional child. The benefit is paid every four weeks. If filers have an annual income level above £50,000, the benefit begins to phase out, and it is no longer made available at an annual income level of £60,000 (*Claim Child Benefit*, 2014).

The United States can look just north to find an example of a country moving from a complex set of child support programs that included, among other things, benefits for hockey lessons, to a much more streamlined system of cash benefits. In 2013, a Trudeau-led Liberal government enacted the Canada child benefit (CCB) of $440 per month for each eligible child under the age of 6 and $370 per month for each eligible child aged 6 to 17 (*Canada Child Benefit (CCB)*, 2021). The change has been popular among parents and helped reduce child poverty. However, some researchers, such as those at the Fraser Institute, argue that this program and others like it are woefully understudied and likely did not have as large an impact as politicians may assert (C. A. Sarlo, 2021).

Pending Proposals

The Biden administration and most congressional Democrats would like to see the expanded Child Tax Credit extended. In early 2022, five Democrat senators sent a letter urging the Biden administration to ensure the expanded CTC is a "centerpiece" of Build Back Better legislation

(Bennet et al., 2022). They heralded the expanded CTC as "a signature domestic policy achievement of this administration" and "an overwhelming success." The letter focused on the anti-poverty merits of the law, from reducing child poverty and hunger, particularly for children of color, to promoting economic growth in rural communities.

Public support for the expanded CTC varies from poll to poll. A Morning Consult and Politico poll in July 2021 found that only 35 percent of respondents said the expansion should "definitely" or "probably" be permanent. On the other hand, a survey from Data for Progress and Mayors for a Guaranteed Income found that 56 percent of voters support making the child tax credit expansions permanent (Schnell, 2021). The monthly payment aspect of the modified policy seems to be popular: three out of four respondents in one study preferred monthly payments rather than a lump-sum annual payment (Hamilton et al., 2021).

Analysts struggle to find explanations for the lukewarm public support shown for the expanded CTC — policies like lowering costs for prescription drugs and expanding Medicare are consistently more popular. Younger Americans, who are more likely to be parents receiving the credit, tend to approve of the expansion, while many older Americans do not. M.I.T. Social policy scholar Andrea L. Campbell suggested that older Americans may hesitate to support benefits that

seem to threaten funding for programs that they rely on heavily, primarily Social Security and Medicare (Campbell, 2002).

Americans may also lump together the expanded CTC as a temporary pandemic program that has no use in a post-covid world, or some may see the full refundability as an unwelcome departure from the traditional means-tested welfare system in the United States. The tepid public response should be noted and analyzed. However, polling is an imperfect gauge of public sentiment, and there is plenty of ongoing research showing the vast benefits of a universal child allowance policy. The New York Times notes that the Affordable Care Act of 2010 did not become consistently popular until 2017 when President Trump and a Republican-held Congress attempted to repeal it (Philbrick, 2022).

With the Expanded Child Tax Credit set to expire at the end of 2021, Congressional Republicans and Democrats debated the best path forward. Most Democrats were in favor of making the expanded child tax credit permanent or at least extended into future years. Some Republicans were also in favor of a larger tax benefit, though the full refundability and lack of a work requirement were major sticking points for conservatives, including Senator Joe Manchin (D-WV), who as a conservative Democrat in a 50-50 Senate would be a major swing vote on any potential legislation, as has been shown in his thwarting of recent pushes by the Democratic

coalition pass the $2 trillion social spending bill known as Build Back Better (Fram, 2021) and, later, to remove the filibuster in order to pass less restrictive federal voting laws (Hulse, 2022). Other conservatives have criticized the use of terminating or curtailing spending programs in the bill earlier than the close of the 10-year budget window, hiding the overall costs. This criticism is valid, though Democrats and Republicans alike have used similar accounting tricks to get a variety of spending bills through Congress palatably throughout history.

In February 2021, Senator Mitt Romney (R-UT) released the Family Security Act, marketed as a Republican alternative to the Expanded Child Tax Credit (Romney, 2021). The Family Security Act would replace the existing Child Tax Credit with a monthly cash benefit for families. The Social Security Administration would distribute $350 per month for children under age six and $250 per month for children under age eighteen.

The phase-out thresholds would remain at $200,000 for single filers (head of household status would be eliminated) and $400,000 for married joint filers. Notably, the benefit would be available to pregnant mothers four months prior to the child's due date.

The bill consolidates a variety of federal policies that currently exist to support families, many of which Romney describes as 'duplicative,' into direct, monthly cash support for families. One

notable change is a modification of the Earned Income Tax Credit (EITC), which is a somewhat complex mechanism for supporting low-income American families, designed to promote working and earning income (*What Is the Earned Income Tax Credit (EITC)?*, 2022). The EITC has come under scrutiny. In part because of the complexity of the EITC, a higher than average percentage of individuals with income below $25,000 are audited every year, as noted in a recent Government

Accountability Office (GAO) report (*Tax Compliance: Trends of IRS Audit Rates and Results for Individual Taxpayers by Income*, 2022).

Under the Family Security Act, the EITC would be simplified to a binary division between a household with dependents and households with no dependents, rather than varying the benefit based upon the number of dependents in the household. Though this change would negatively impact some families at certain income levels with certain numbers of dependents, the Family Security Act promises that no family will receive less money than before this change. The details on how that will be accomplished are scarce and will need to be clarified in future discussions. Still, this change may help simplify the tax filing procedure for low-income Americans and reduce IRS audits and improper payments among the lowest-income Americans.

Eliminating the head of household designation as

a tax filing option is not necessarily the headlining feature of the plan, but it would be an impactful and controversial change. Conservatives have long advocated for eliminating "marriage penalties" in the tax code, arguing that policy decisions should support working families while encouraging marriage as a pathway to escape poverty (Roth, 2021). Head of household status is available to unmarried people who have a dependent and pay most of the costs of maintaining the household wherein they and the dependent live for more than six months in the year. In 2015, 22 million people filed as head of household. The status results in a variety of favorable tax preferences, most notably that lower tax rates apply to a greater share of income earned by heads of households compared to those who file as single (*Eliminate or Modify Head-of-Household Filing Status*, 2018). For example, the expanded Child Tax Credit began to phase out at income levels of $75,000 for single filers and $150,000 for joint filers but at an income level of $112,500 for head of household filers. For many families, these realities disincentivize marriage. However, as the National Women's Law Center and others have argued, eliminating head of household status would negatively impact single parents and other unmarried people who are supporting dependents, particularly low- and middle-income people (*Eliminating the Head of Household Filing Status Would Hurt Women*, 2017).

Benefit cliffs (income levels where families no longer qualify for the credit) remain at the higher TCJA levels. To balance out the additional costs of the larger family benefits, the plan would eliminate the State and Local Tax Deduction (SALT), which Romney describes as "an inefficient tax break to upper-income taxpayers." This is a valid criticism. Democratic support of the SALT deduction, even attempting recently to eliminate the deduction cap of $10,000 a year or raise it dramatically, is a strange outlier to the party's general platform supporting higher taxes on the rich. In 2016, 77 percent of SALT deduction benefits went to those with incomes above $100,000; only 6.6 percent accrued to taxpayers making less than $50,000 per year (Bellafiore, 2021). The Brookings Institution called the SALT tax deduction "a handout to the rich" and argued that it should be eliminated, not expanded (Pulliam & Reeves, 2022). If Congress repealed the cap, 96 percent of the benefits would go to the top quintile (an average tax reduction of $2,640), 57 percent would benefit the top one percent (a cut of $33,100), and 25 percent would benefit the top 0.1 percent (a reduction of nearly $145,000) (*T20-0182*, 2020).

Romney argued that the plan would establish a firm national commitment to all of America's families while cutting American child poverty by up to one-third and immediately raising nearly 3 million children out of poverty. While, on the surface, a plan to provide unconditional cash benefits to the parents of children seems antithetical to traditional fiscal conservatism, there are aspects of the plan that appeal to many in the Republican ranks. The unprecedented expansion of eligibility to include unborn children may appeal to pro-life

conservatives. Eliminating the Head of Household filing option would remove the tax disincentive for marriage for parents raising children — the phase-out threshold would not be higher for single filers than for married filers. The proposal's plans to reform and consolidate "antiquated" federal programs could also gain traction among fiscal conservatives.

The central sticking point with this plan for many Republican senators (and Senator Joe Manchin) is the lack of a work or income requirement for receiving the benefit. Because the plan is fully refundable, even those who do not receive an income are entitled to the Child Tax Credit. Senators Rubio (R-FL) and Lee (R-UT) offered a counterproposal in the form of an amendment to the Senate Budget Resolution to expand the Child Tax Credit. Their proposal would expand the Child Tax Credit while ensuring the credit does not go to individuals who are not working (Rubio et al., 2021). Senator Rubio said of the Biden Administration's plans to make permanent the child tax credit, "This new Government Child Allowance scraps incentives for marriage, destroys the child-support enforcement system, and abandons requirements for work. We've seen the destructive consequences that follow when the government pays people not to work." Senator Lee asserted, "To have a truly pro-family tax code, we must have a pro-growth and pro-worker tax code,

creating monthly welfare payments under the Government

Child Allowance, however, will only disincentivize marriage and the responsibility of parents to provide for their families."

MOVING FORWARD

A few principles should guide discussions of the future of the Child Tax Credit.

First, providing a child allowance is an investment with a high rate of return for domestic prosperity. Second, the Child Tax Credit must be easy to navigate for taxpayers and more efficient to administer for government agencies. Third, making the payments monthly and fully refundable are vital steps to ensure the positive impact of the program on children and families.

Priority One: Sufficient Support

First, providing a child allowance is an investment with a high rate of return for domestic prosperity. Congress should pursue the development of a policy targeted at supporting children, those in our society that are some of the most vulnerable but also have the most ahead of them, that generates a $7 overall return for every $1 spent. The benefit should be substantial — a baseline of $3,000 per year per child, with an additional benefit for children under six, seems to be an ideal and politically feasible place to start discussions.

There are two income benefits available for families that cannot find work or whose income is too low to qualify for work-related tax credits: the Supplemental Nutrition Assistance Program (SNAP) and the Temporary Assistance for Needy Families (TANF) program. SNAP provides a vital

baseline of support for families with children, but expenses are limited to food purchases and cannot be used for other essential living expenses such as housing and utilities. TANF is a cash assistance program established as part of the welfare reform of 1996. Over time, its impact has dwindled as the program has not been sufficiently modernized: the number of families receiving cash assistance per 100 families in poverty has declined from 68 in 1996 to 23 in 2015 (Floyd et al., 2017). These programs are insufficiently supportive for low-income families, and a generous child allowance is needed to help children raised in these families avoid unfortunate life outcomes.

Priority Two: Monthly, Refundable

Second, making the payments monthly and fully refundable are vital steps to ensure the positive impact of the program on children and families. Studies have found that unconditional cash transfers have significant impacts on economic outcomes and psychological well-being, as people feel confident in the stream of income they will be receiving and can better plan for advancing in life. Further, parents are more likely to spend monthly cash transfers on essentials like food and utilities than they are with large, annual transfers. Families have understandable difficulties budgeting for their end-of-year lump-sum EITC or CTC benefits because rent is due monthly, and groceries are purchased weekly. Thus, lump-sum annual transfers are more likely to be spent on durable

goods and major purchases (Haushofer & Shapiro, 2016).

While work requirements and means-testing can be well-intentioned, and perhaps necessary for certain types of benefits, lawmakers must take care to avoid creating overly complex programs that end up reinforcing rather than tearing down barriers to socioeconomic mobility (Waxman & Hahn, 2021). Instead, the goal of the welfare system should be to efficiently and effectively support the vulnerable while removing disincentives for work and income growth. A child allowance that is predictable, fully refundable, and with higher, slow phase-out levels can best accomplish those priorities.

Priority Three: Effectively Administered

Finally, the Child Tax Credit must be more effectively administered than it is currently. The government should provide predictable, simple payments to families through established channels, such as the IRS. The complex nature of the United States' welfare programs results in decreased productivity and time loss (Thompson, 1994).

Rather than promoting work and socioeconomic mobility, this reality may make finding work more difficult for low-income individuals, deepening dependence on government support. More research should be conducted in this area to get a better sense of the magnitude of efficiency loss caused by strictly means-tested social welfare programs like those in the United States.

Additionally, the programs tend to miss the people who need the benefits the most. The Temporary Assistance for Needy Families (TANF) program is a federal block grant provided to states to fund their family and child support programs. During the COVID-19 pandemic, just 21 percent of low-income families received TANF benefits in 2020, compared to 68 percent in 1996 (*Policy Basics: Temporary Assistance for Needy Families.*, 2022).

Further, the complex requirements create more administrative burdens for already overstretched government agencies. The federal government loses about $600B to tax fraud and underpayments every year. This "tax gap" will cost the government $7 trillion in lost revenue over the next decade — and it's mostly the wealthy that can avoid paying their full tax bill. Researchers estimate that the top one percent of earners account for 28 percent of the unpaid taxes in the United States (Treasury, 2021). The IRS needs more staffing and updated technology; they do not need more complex tax and welfare systems that force them to spend inordinate amounts of time auditing those in the lowest income brackets.

A Reasonable Path Forward

Senator Romney's Family Security Act and the American Rescue plan's expanded Child Tax Credit have enough common priorities and policies that the plans should be used as the baseline for negotiations moving forward. Conservative

priorities, such as minimizing the marriage penalty in the tax code and reducing the impact on the federal deficit, do not destroy hope for a robust child allowance system that supports low- and middle-income families. Legislators should ensure that those impacted by a major change in the tax code, such as modification of the EITC or removal of the head of household status (particularly for single parents), do not see their benefits or support drop dramatically. If Congress does not take care, these decisions can result in instability for millions of households.

If work requirements must be introduced for political feasibility, the requirements should be simple and easy to navigate. A proposal that may work to appease conservatives would be a simple proof of work with a W-2 form or other documentation within a recent period, perhaps two years. Those out of work for reasons outside of their control, actively searching for a job, or focused on raising their children will still receive the full benefit. Parents will have the peace of mind that a base level of income will be there to help provide the essentials for their children.

CONCLUSION

In a time of global economic uncertainty, the time is now to strengthen our support for parents, especially those of lower- and middle-income. The ultimate aim of a child allowance policy should be twofold: 1) reduce child poverty and 2) encourage

Americans to bear and raise children in stable homes. A balanced consideration of liberal and conservative values and policy priorities can engender a solution that accomplishes these goals. Converting the Child Tax Credit into a highly refundable monthly child allowance will reduce child poverty, promote social stability, and generate economic dynamism and growth. America's children deserve solutions, not legislative bickering and inaction.

REFERENCES

Ananat, E., Glasner, B., Hamilton, C., & Parolin, Z. (2021). *Effects of the Expanded Child Tax Credit on Employment Outcomes*. Columbia University Center on Poverty and Social Policy.

Baird, S., McKenzie, D., & Özler, B. (2018). The effects of cash transfers on adult labor market outcomes. *IZA J Develop Migration*, *8*, 22. https://doi.org/10.1186/s40176-018-0131-9

Bellafiore, R. (2021). *Salt deduction: Who benefits from the state and local deduction? Tax Foundation*. Tax Foundation. https://taxfoundation.org/salt-deduction- benefit/

Bennet, M. F., Brown, S., Booker, C. A., Warnock, R., & Wyden, R. (2022, January 26). *Senate Democrats Letter to President Biden on the Child Tax Credit*. https://s3.documentcloud.org/documents/21186217/senate-democrats-child-tax- credit-letter-to-biden-and-harris.pdf

Browning, M. (1992). Children and Household Economic Behavior. *Journal of Economic Literature*, *30*(3), 1434–1475. http://www.jstor.org/stable/2728065

Campbell, A. L. (2002). Self-Interest, Social Security, and the

Distinctive Participation Patterns of Senior Citizens. *The American Political Science Review*, *96*(3), 565– 574.

Canada child benefit (CCB). (2021, May 17). Canada. https://www.canada.ca/en/revenue-agency/services/child-family-benefits/canada-   child-benefit-overview/canada-child-benefit-we-calculate-your-ccb.html

Chang, C., & Madrick, J. (2016). Investing in our kids using one simple tool: Cash. In *The Century Foundation*. https://tcf.org/content/commentary/investing-in-our- kids-using-one-simple-tool-cash/

Claim   child   benefit.   (2014).   United   Kingdom. https://www.gov.uk/child-benefit   Crandall-Hollick,   M.   L. (2021). The Child Tax Credit: Legislative History.

Congressional Research Service, *R45124*. https://sgp.fas.org/crs/misc/R45124.pdf

Eliminate or modify head-of-household filing status. (2018). Congressional   Budget   Office.   https://www.cbo.gov/budget–options/54789

Eliminating the head of household filing status would hurt women.   (2017).   National   Women's   Law   Center. https://nwlc.org/wp-content/uploads/2017/08/Eliminating-the-Head-of-Household-Filing-Status-Would-Hurt-Women.pdf

Fertility rate, total (births per woman)—United States. (2022). World   Bank.   https://data.worldbank.org/indicator/SP.DYN.TFRT.IN?locations=US

Floyd, I., Pavetti, L., & Schott, L. (2017). *TANF Reaching Few Poor Families*. Center on Budget and Policy Priorities.

Fram, A. (2021). *Manchin not backing Dems' $2T bill, potentially dooming it*. AP NEWS. https://apnews.com/article/manchin-rejects-biden-spending-bill-   democrats-election-e7a0ca4c25c686e91cb21e166c44821b

Garfinkel, I., Sariscsany, L., Ananat, E., Collyer, S., & Wimer,

C. (2021). *The Costs and Benefits of a Child Allowance*. Center on Poverty and Social Policy at Columbia University. https://static1.squarespace.com/static/ 610831a16c95260dbd68934a/t/6113e84ec4ea72172c221d2c/ 1628694621357/Child-Allowance-CBA-discussion-paper- CPSP-2021.pdf

Gleckman, H. (2022). *Why Congress really is fighting over the refundable Child Tax Credit*. Forbes. https://www.forbes.com/ sites/howardgleckman/2022/01/28/why- congress-really-is-fighting-over-the-refundable-child-tax-credit/

González, L., & Trommlerová, S. K. (2021). Cash Transfers and Fertility: How the Introduction and Cancellation of a Child Benefit Affected Births and Abortions. *Journal of Human Resources*, 0220-10725R2. https://doi.org/10.3368/ jhr.59.1.0220-10725R2

Hamilton, L., Roll, S., Despard, M., Maag, E., & Chun, Y. (2021). *Employment, Financial and Wellbeing Effects of the 2021 Expanded Child Tax Credit: Wave 1 Executive Summary*. Social Policy.

Hammond, S. (2021). *Measuring the Child Tax Credit's Economic and Community Impact*. Niskanen Center. https://www.niskanencenter.org/report-measuring-the- child-tax-credits-economic-and-communityimpact/.

Haushofer, J., & Shapiro, J. (2016). The short-term impact of unconditional cash transfers to the Poor: Experimental Evidence from Kenya. *The Quarterly Journal of Economics*, *131*(4), 1973–2042. https://doi.org/10.1093/qje/qjw025

Holzer, H. J., Whitmore Schanzenbach, D., Duncan, G. J., & Ludwig, J. (2008). The economic costs of childhood poverty in the United States. *Journal of Children and Poverty*, *14*(1), 41–61. https://doi.org/10.1080/10796120701871280

Hulse, C. (2022). After a day of debate, the voting rights bill

is blocked in the Senate. *The New York Times*. https://www.nytimes.com/2022/01/19/us/politics/senate-voting- rights-filibuster.html

Jabbari, J., Hamilton, L., Roll, S., & Grinstein-Weiss, M. (2021). *The new child tax credit does more than just cut poverty*. Brookings Institution. https://www.brookings.edu/blog/up–front/2021/09/24/the-new-child-tax-credit- does-more-than-just-cut-poverty/

Laroque, G., & Salanié, B. (2005). *Does Fertility Respond to Financial Incentives?* (CEPR Discussion Paper No. 5007). C.E.P.R. Discussion Papers. https://econpapers.repec.org/paper/cprceprdp/5007.htm

Lino, M. (2015). Expenditures on children by families. *Dept. of Agriculture*. https://fns- prod.azureedge.us/sites/default/files/resource-files/crc2015-march2017.pdf

Matthews, D. (2016, May 23). Sweden pays parents for having kids—And it reaps huge benefits. Why doesn't the US? *Vox*. https://www.vox.com/2016/5/23/11440638/child-benefit-child-allowance

Morgan, S. P. (2003). Is low fertility a twenty-first-century demographic crisis? *Demography*, *40*(4), 589–603. https://doi.org/10.1353/dem.2003.0037 Nikulina, V., Widom, C. S., & Czaja, S. (2011). The role of childhood neglect and childhood poverty in predicting mental health, academic achievement and crime in adulthood. *American Journal of Community Psychology*, *48*(3–4), 309–321. https://doi.org/10.1007/s10464-010-9385-y

Parolin, Z., Sophie, C., & Curran, M. A. (2022). Absence of Monthly Child Tax Credit Leads to 3.7 Million More Children in Poverty in January 2022. *Poverty and Social Policy Brief*, *6*(2). www.povertycenter.columbia.edu/publication/monthly- poverty-january-2022

Philbrick, I. P. (2022). Why isn't Biden's expanded Child tax credit more popular? *The New York Times.* https://www.nytimes.com/2022/01/05/upshot/biden-child-tax- credit.html

Policy basics: Temporary assistance for needy families. (2022). Center on Budget and Policy Priorities. https://www.cbpp.org/research/family-income- support/temporary-assistance-for-needy-families

Pulliam, C., & Reeves, R. V. (2022). *The Salt Tax Deduction is a handout to the rich.* Brookings Institution. https://www.brookings.edu/blog/up–front/2020/09/04/the-salt-tax-deduction-is-a-handout-to-the-rich-it-should-be-eliminated-not-expanded/

Raphael, D. (2011). Poverty in childhood and adverse health outcomes in adulthood. *Maturitas*, *69*(1), 22–26. https://doi.org/10.1016/j.maturitas.2011.02.011 Rector, R., & Hall, J. B. (2021). Government supports would grow to $76,400 per poor family. *The Heritage Foundation.* https://www.heritage.org/welfare/commentary/government-supports-would-grow- 76400-poor-family

Romboy, D. (2021). How Mitt Romney proposes to encourage Americans to create families, have children. *Deseret News.* https://www.deseret.com/utah/2021/3/23/22346532/how-mitt-romney-proposes- encourage-americans-create-family-have-chidlren-ross-douthat

Romney, S. M. (2021). *The Family Security Act.* https://cdn.vox- cdn.com/uploads/chorus_asset/file/22279576/family_security_act_one_pager_appendix.pdf

Rostad, W. L., Klevens, J., Ports, K. A., & Ford, D. C. (2020). Impact of the United States federal

child tax credit on childhood injuries and behavior problems. *Children and Youth Services Review*, *109*, 104718.   https://doi.org/10.1016/j.childyouth.2019.104718

Roth, P. (2021). Reducing the marriage penalty for low-income families. *American Enterprise Institute*. https://www.aei.org/poverty-studies/reducing-the-marriage- tax-penalty-for-low-income-families/

Rubio, Florida, U. S. S., & Rubio, M. (2021). *Lee Push Pro-Work child tax credit amendment.* https://www.rubio.senate.gov/public/index.cfm/2021/8/rubio-lee- push-pro-work-child-tax-credit-amendment

Sarlo, C. (2013). The Cost of Raising Children (August 22, 2013. *Fraser Institute*. https://ssrn.com/abstract=2318106

Sarlo, C. A. (2021). *Does the Canada Child Benefit actually reduce child poverty?* https://fraserinstitute.org/sites/default/files/does-the-canada-child-benefit-   actually-reduce-child-poverty.pdf

Schnell, M. (2021). Majority in new poll says expanded Child Tax Credit should not be permanent. *The Hill*. https://thehill.com/policy/finance/564063-majority-in-new-poll-says-expanded-child-tax-credit-should-not-be-permanent/

Shabad, R., Haake, G., V, T., F., & Tsirkin, J. (2021). Manchin privately raised concerns that parents would use child tax credit checks on drugs. *NBCNews.Com*.
 https://www.nbcnews.com/politics/congress/manchin-privately-raised-concerns-   parents-would-use-child-tax-credit-n1286321

Skiera, A. (2022, October). *Personal independence behind declining birth rates.* Harris Poll. https://theharrispoll.com/briefs/birth-rates/

Sterling, J., Jost, J. T., & Hardin, C. D. (2019). Liberal and conservative representations of the good society: A (social) Structural Topic Modeling Approach. *SAGE Open*, *9*(2), 215824401984621. https://doi.org/10.1177/2158244019846211 *T20-0182*.

(2020). Tax Policy Center. https://www.taxpolicycenter.org/model- estimates/ health-and-economic-recovery-omnibus-emergency-solutions-heroes- act-may-2020/t20-7

Tax compliance: Trends of IRS audit rates and results for individual taxpayers by income. (2022). U.S. Government Accountability Office. https://www.gao.gov/products/gao-22-104960

Thompson, L. H. (1994). The Advantages and Disadvantages of Different Social Welfare Strategies. *Social Security Bulletin*, *57, Ser. 3.* https://www.ssa.gov/policy/docs/ssb/v57n3/ v57n3p3.pdf

Treasury, U. S. D. (2021). *The case for a robust attack on the Tax Gap.* https://home.treasury.gov/ news/featured-stories/the-case-for-a-robust-attack-on- the-tax-gap

Troller-Renfree, S. V., Costanzo, M. A., Duncan, G. J., Magnuson, K., Gennetian, L. A., Yoshikawa, H., Halpern-Meekin, S., Fox, N. A., & Noble, K. G. (2022). The impact of a poverty reduction intervention on infant brain activity. *Proceedings of the National Academy of Sciences*, *119*(5). https://doi.org/ 10.1073/pnas.2115649119

Waxman, E., & Hahn, H. (2021). Work requirements sound

good, but the evidence just doesn't support them. *Urban Institute.* https://www.urban.org/urban-wire/work-requirements-sound-good-evidence-just-doesnt-support-them

What is the earned Income Tax Credit (EITC)? (2022). Tax Foundation. https://taxfoundation.org/tax-basics/earned-income-tax-credit-eitc/

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131. **A Continuous 60,000 Year Sediment Record Documenting Abrupt to Procession-Scale Climate Change and Ecosystem Response at Fish Lake Utah, Upper Colorado River Basin**

Margot Langue; Andrea

Brunelle (Geography); Anna Petersen; Vachel Carter; Haley Segura; Maya Upton; Jesse Morris; and Mitchell Power (Geography)

Faculty Mentor: Andrea Brunelle (Geography, University of Utah)

Fish Lake, Utah's geographic location provides a unique opportunity to explore the critical role of wildfire in high-elevation forested ecosystems in the Colorado Plateau. In light of extreme drought conditions in the western United States, we are investigating the linkages among vegetation communities, climate change and natural and anthropogenic disturbance. In addition, this study site is located proximal to the ancient "Pando" aspen clone, where we are studying this ancient clone's specific history (believed to be 60,000 years old), and the overall aspen forest response to climate- and fire-driven events throughout the Holocene. This research demonstrates that wildfires have happened continuously throughout the Holocene, with a period of maximum fire frequency occurring during the last two millennia. In contrast, the early Holocene was a time of reduced fire activity, longer fire-return intervals and protracted climate-driven drought in response to seasonal forcing from maximum summer insolation. Pollen evidence suggests aspen persisted throughout the Holocene with higher and lower relative pollen abundance linked to increases and decreases in fire magnitude, respectively. For example, the

largest fire episodes of the Holocene occurred ~4700 cal yr BP and was associated with an increase in aspen pollen within the Fish Lake record. This research is part of a larger timeline, exploring climate, vegetation and fire dynamics during Marine Isotope Stage 3 (25-60,000 cal yr BP) and is designed to provide land managers new insights into the long-term role of disturbances in aspen forests and potentially offer recommendations for future maintenance of healthy aspen ecosystems.

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132. **Research Reflection by Margot Langue**

Margot Langue

Faculty Mentor: Andrea Brunelle (Geography, University of Utah)

My undergraduate research involvement has unquestionably been one of the most formative experiences of my entire undergraduate education. I first joined the Records of Environment and Disturbance Lab in December of 2021, working with Dr. Andrea Brunelle and Dr. Mitchell Power on a National Science Foundation funded project. We examined the wildfire history of the high-elevation forested ecosystem of Fish Lake, Utah. My position as an undergraduate research analyst provided me with strong footing in paleoclimatological proxy record analysis and allowed me to develop my prior laboratory experience specific to my future career goals. My research motivated me to seek out new opportunities in the realm of paleoclimatology, out of excitement to learn as much

as possible, granting me an internship with The Natural History Museum of Utah. This internship allows me to explore and research climate records through the analysis of other proxy records, which would not have been possible without my prior undergraduate charcoal analysis research experience. My charcoal analysis research position has also granted me the ability to explore different areas of research, permitting me to focus my career goals under the guidance and expertise of my mentors. I am incredibly grateful for my undergraduate research experience, and everything it has prepared me for in my future academic and career path.

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133. **Associations Among Maternal Trauma History, Prenatal Emotion Dysregulation, and Prenatal Sleep Quality**

Marissa Larkin

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ABSTRACT

Sleep is an increasingly recognized correlate and predictor of long-term mental and physical health. Those who have endured traumas throughout their lifespan may experience mental and physical health difficulties, including poor sleep quality. One

group of individuals who are especially vulnerable to experiencing poor sleep quality are pregnant women, especially those who have experienced trauma. The overarching aim of this study was to examine how maternal trauma history may be related to sleep quality during pregnancy. We also examined if the relation between maternal trauma history and sleep quality varied across levels of emotion dysregulation, given our hypothesis that emotion regulation skills may buffer the effects of trauma on sleep quality. Eighty-six 3rd-trimester pregnant women aged 19-38 completed self- report measures pertaining to traumatic life experiences, emotion dysregulation, and subjective sleep quality. Hierarchical linear regression models revealed that higher levels of emotion dysregulation predicted poorer sleep quality during pregnancy. Maternal trauma history did not predict prenatal sleep quality, nor did this relation vary across levels of emotion dysregulation. Given that poor prenatal sleep has been associated with negative maternal postnatal health outcomes, offspring development, and maternal-infant relationships, our study highlights the utility of improving emotion regulation skills during pregnancy as a means for also improving sleep quality. The current study is one of few to examine emotion dysregulation during pregnancy and provides additional evidence that it may be an important factor for identifying mental health concerns during pregnancy.

Keywords: emotion dysregulation, pregnancy, sleep quality, trauma

INTRODUCTION

Adequate sleep is critical for mental and physical health across the lifespan (Alvarez & Ayas, 2004; Clement-Carbonell

et al., 2021; Medic et al., 2017). Consequences of poor sleep can include diabetes, heart attack, depression, and anxiety (Clement-Carbonell et al., 2021; Colten & Altevogt, 2006; Medic et al., 2017). One group of individuals who are especially vulnerable to experiencing poor sleep are those who are pregnant (Facco et al., 2010; Hedman et al., 2002; Kennedy et al., 2007; Mindell & Jacobson, 2000; Mindell et al., 2015; Sedov et al., 2018).

Numerous biological and anatomical changes during pregnancy confer risk for poor sleep (Fernández-Alonso et al., 2012; Hedman et al., 2002; Kennedy et al., 2007; Kizilirmak et al., 2012; Mindell et al., 2015; Sedov et al., 2018); however, not all pregnant people experience the same severity of sleep disturbances. Why do some pregnant people experience *especially* poor sleep quality?

Poor prenatal sleep has been associated with various aspects of maternal postnatal health, including higher risk of postpartum depression (Chang et al., 2010; Okun et al., 2009; Pietikäinen et al., 2018), gestational diabetes (Facco et al., 2017), and greater and longer lasting weight gain (Sharkey et al., 2016). Poor prenatal sleep quality has also been linked to effects on offspring development, such as a higher risk of preterm birth (Micheli et al., 2011), low birth weight (Plancoulaine et al., 2017), difficulties with sleep throughout childhood (Armstrong et al., 1998), delay of gross motor and language development (Li et al., 2023), poor executive function (Lahti-Pulkkinen et al., 2018), and a general increased risk of psychiatric problems and poor neurodevelopment (Lahti-Pulkkinen et al., 2018). As for maternal-infant relationships, poor prenatal sleep quality has been associated with a higher risk of infant negative reactivity (Ciciolla et al., 2022), maternal and infant sleep problems (Ciciolla et al., 2022), difficulty with breastfeeding (Gordon et

al., 2021), maternal fatigue (Pires et al., 2010), postpartum depression

(Chang et al., 2010; Okun et al., 2009; Pietikäinen et al., 2018; Pires et al., 2010), and insecure mother-child attachment security (Newland et al., 2016). Given that poor prenatal sleep has been associated with these outcomes, it is important to identify risk and protective factors for prenatal sleep health. Clarifying these susceptibility factors will help to inform intervention efforts for pregnant people in greatest need of those supports.

Maternal Trauma History and Prenatal Sleep Quality

Sleep disturbances and symptoms indicative of clinical sleep disorders increase significantly across the duration of pregnancy (Facco et al., 2010; Hedman et al., 2002; Kennedy et al., 2007; Mindell & Jacobson, 2000; Mindell et al., 2015; Sedov et al., 2018). Subjective sleep quality, specifically, may be poor as early as the 1st trimester of pregnancy and worsens substantially by the 3rd trimester due to frequent urges to urinate, restless legs, physical discomfort, snoring, and ruminations about childbirth and parenting (Fernández-Alonso et al., 2012; Hedman et al., 2002; Kalmbach et al., 2020; Kennedy et al., 2007; Kizilirmak et al., 2012; Mindell et al., 2015; Sedov et al., 2018). In addition to these physical factors affecting sleep quality, research has indicated that women's experiences with trauma across the lifespan influence prenatal sleep (Gelaye et al., 2015; Miller-Graff & Cheng, 2017; Nevarez-Brewster et al., 2022; Sanchez et al., 2016; Takelle et al., 2022). Both childhood abuse—particularly, physical and sexual abuse—and intimate partner violence during adulthood have been associated with poor sleep quality during pregnancy (Gelaye et al., 2015; Miller-

Graff & Cheng, 2017; Nevarez-Brewster et al., 2022; Sanchez et al., 2016; Takelle et al., 2022). The association between maternal trauma history and prenatal sleep quality may be due to psychological factors, such as depression and ongoing trauma symptoms during adulthood (Gelaye et al., 2015). In addition, this association may be due to the fact that traumatic life experiences can alter the hypothalamic-pituitary-adrenal (HPA) axis, which also is involved in the regulation of sleep (Buckley & Schatzberg, 2005; Heim et al., 2008). Specifically, chronic trauma exposure can increase HPA axis hyperactivity and cortisol production, which then can contribute to increased awakenings, sleep fragmentation, and decreased slow-wave sleep (Buckley & Schatzberg, 2005; Heim et al., 2008).

Emotion Dysregulation

Chronic and repeated trauma exposure also can have adverse effects on development that compromise emotion regulation abilities (Heleniak et al., 2015; Jenness et al., 2020; Kerig, 2018). Similar to how trauma may alter HPA axis activity, traumatic life experiences have been found to alter the limbic HPA axis, which facilitates emotion regulation (Kerig, 2018). Difficulties with emotion regulation, or emotion dysregulation, may be characterized by emotional experiences and expressions that are intense and interfere with interpersonal functioning and goal-directed behaviors (Crowell et al., 2020). Emotion dysregulation involves any combination of limited awareness, understanding, and acceptance of

emotions; poor ability to control impulsive behaviors when upset; and limited capacity to regulate emotions in order to achieve one's goals (Gratz & Roemer, 2004). Emotion dysregulation as a result of childhood trauma has been associated with numerous psychopathologies and physical health risks, such as depression, anxiety, borderline personality disorder, cardiovascular disease, type II diabetes, arthritis, and Alzheimer's disease (Appleton et al., 2011; Ball et al., 2012; Crowell et al., 2015; Folk et al., 2014; Hofmann et al., 2012; Kerig, 2018; Kiecolt-Glaser et al., 2002; Miranda et al., 2013). Among pregnant women, emotion dysregulation was found to be associated with depression, anxiety, borderline personality disorder, and self-injurious thoughts and behaviors (Lin et al., 2019). Disturbed sleep is a key feature of many of these psychopathologies, and indeed, there is empirical evidence of associations between emotion dysregulation and poor sleep among non-pregnant adults (Ennis et al., 2017; Mauss et al., 2013; Palmer & Alfano, 2017). However, there is limited research on associations among trauma history, emotion dysregulation, and sleep quality among pregnant women, specifically.

The Current Study

To advance understanding of factors that influence sleep quality during pregnancy, we examined if maternal trauma history predicted prenatal sleep quality, and if the relation between

maternal trauma history and prenatal sleep quality varied based on levels of emotion dysregulation. We hypothesized that more lifetime experiences with trauma would predict poorer prenatal sleep quality. Furthermore, we hypothesized that the relation between trauma history and sleep quality would not be the same across levels of emotion dysregulation. Specifically, we hypothesized that trauma history would predict poorer prenatal sleep quality among those who also reported high levels of emotion dysregulation, and the relation between trauma history and sleep quality would be nonsignificant among those who reported low levels of emotion dysregulation. This latter part of the hypothesis would suggest that effective emotion regulation abilities can buffer the effects of trauma on sleep quality.

METHOD

Participants

Participants (N = 86; Mage = 29.42, SD = 4.44 years) included 3rd-trimester pregnant women enrolled in a longitudinal study on sleep and emotion dysregulation during the perinatal period (R01MH119070, MPIs Crowell & Conradt; F31MH124275, PI Kaliush). Eligibility criteria included: (1) 18 to 40 years of age, (2) singleton pregnancy, (3) no present illicit substance use, and (4) no severe pregnancy complications (e.g., gestational diabetes, preeclampsia). All participants identified as women. The majority of participants

identified as White (67.4%), and other racial identities included more than one race (9.3%), Asian (8.1%), Black or African American (4.7%), and Native Hawaiian or other Pacific Islander (1.2%). Some participants declined to report their race (5.8%), and others preferred to self-report their race (3.5%; e.g., Mexican, Middle Eastern). Approximately 25% of the sample identified as Hispanic or Latina, and 68.6% had been pregnant at least one other time before participating in the study. The sample was socioeconomically diverse, with the annual household income ranging from less than $5,000 (3.5%) to $250,000 or more (2.3%) and the most frequently reported income range being $75,000 to $99,999 (16.3%). The educational backgrounds of the sample include a high school degree or less (12.8%), some college but no degree (17.4%), technical school or associate's degree (16.2%), bachelor's degree (33.7%), or master's or doctoral degree (19.8%; see Table 1 for additional demographics).

Procedure

All study procedures were approved by the University of Utah Institutional Review Board. The present study sample size ($N = 86$) aligned with that which was predetermined using regression-based power estimates. Participants were recruited and enrolled to achieve a uniform distribution on a measure of emotion dysregulation to yield a sample with a wide range of distress and emotion

regulation difficulties. Researchers recruited pregnant women from various obstetrics and gynecology clinics within the University of Utah Healthcare system. Prior to recruitment, the women were screened via their online medical records to exclude those who already met specific exclusion criteria. Interested and eligible pregnant women were then contacted by study staff to coordinate participation during their 3rd trimester of pregnancy.

Participation included online questionnaires assessing demographics, psychopathology, and trauma history, as well as seven consecutive daily surveys assessing sleep health. All questionnaires were completed via Research Electronic Data Capture (REDCap; https://www.project-redcap.org/) and Qualtrics (http://www.qualtrics.com), both of which are secure web applications. Participants were financially compensated with online gift cards.

Measures

Emotion Dysregulation. The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004) is a 36-item self-report measure designed to assess various aspects of emotional dysregulation—namely, difficulty engaging in goal-directed behaviors, impulse control difficulties, lack of emotional awareness, nonacceptance of emotional responses, limited access to emotion regulation strategies, and lack of emotional clarity. Sample items include, "When I'm upset, I become out of control" and "When I'm upset, I believe I will

stay that way for a long time". Participants respond to these items with a five-point Likert scale ranging from 1 (*Almost Never*) to 5 (*Almost Always*). Total scores are summed and can range from 36 to 180; higher scores indicate greater difficulty with emotion regulation. In the present sample, the DERS total score demonstrated high internal consistency (α = .96).

Maternal Trauma History. The Traumatic Experiences of Betrayal across the Lifespan (TEBL; Kaliush et al., under review) assesses maternal trauma history. The TEBL examines numerous components of 22 distinct traumatic life experiences, including the accumulation, frequency, chronicity, and developmental timing of those potential traumas. Traumatic life experiences assessed by the TEBL are primarily interpersonal in nature and align with life experiences recognized as traumatic by the *International Statistical Classification of Diseases and Related Health Problems*, 11th edition (ICD-11; World Health Organization, 2019) and *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition (DSM-5; American Psychiatric Association, 2013). The measure comprises yes-or-no questions, typically preceded by the prompt, "At any time in your life, has someone with whom you were close or trusted…," followed by an item such as "…ignored you or withheld praise or affection to make you feel badly about yourself?" If participants endorsed a traumatic life experience, they were prompted to indicate approximately how many times they experienced that trauma during different developmental stages: infancy to early childhood (0-5), middle childhood (6-11), adolescence (12-17), adulthood (18+), age 18 to their current pregnancy, and the 1st, 2nd, and 3rd trimesters of their current pregnancy. In the present study, maternal trauma history was represented by a score ranging from 0

to 22 reflecting the accumulation of trauma across the lifespan, or the number of distinct traumatic life experiences the participant endorsed.

Subjective Sleep Quality. The Consensus Sleep Diary (CSD; Carney et al., 2012) is a standardized self-monitoring tool that assesses numerous sleep health parameters, including sleep onset latency, wake after sleep onset, and subjective sleep quality. Over the course of seven consecutive mornings, participants answered questions pertaining to their previous night's sleep, including ratings of their sleep quality ranging from 1 (*Very poor*) to 5 (*Very good*). Participants' daily sleep quality ratings were averaged to obtain one score reflecting overall subjective sleep quality during their 3rd trimester of pregnancy.

Covariates. Participants' age and parity were included as covariates. Age was included because of empirical evidence that sleep quality deteriorates with increasing age, especially among women (Madrid-Valero et al., 2017). Parity is commonly included as a covariate in perinatal research because it is theorized that women in their first pregnancy may experience stressors and psychopathology differently than do women who were previously pregnant one or more times (Narayan et al., 2018).

Analytic Plan

Preliminary Analyses. Statistical analyses were conducted using SPSS Statistics version 27 (IBM

Corp, 2020). Descriptive analyses were run to determine demographic information as well as the range of values for maternal trauma history, emotion dysregulation, and prenatal sleep quality. We also ran Pearson bivariate correlation analyses to examine associations among demographic variables, maternal trauma history, emotion dysregulation, and prenatal sleep quality. No data were missing. We grand-mean-centered the continuous predictor variables (i.e., maternal trauma history and emotion dysregulation) to account for potential multicollinearity when creating an interaction term. We ensured that the outcome variable, prenatal sleep quality, met assumptions of normality in order to run linear regression models.

Primary Analyses. We ran hierarchical linear regression models to test maternal trauma history and emotion dysregulation as predictors of prenatal sleep quality. In block 1 of the model, we included planned covariates (i.e., parity and age). In block 2, we included maternal trauma history and emotion dysregulation. Finally, in block 3, we included the interaction between maternal trauma history and emotion dysregulation. This analytic procedure revealed if the interaction between maternal trauma history and emotion dysregulation predicted prenatal sleep quality above and beyond the main effects and covariates.

RESULTS

Bivariate Correlations (Table 2)

First, correlation analyses revealed a significant association between parity and prenatal sleep quality, $r(84) = -.22$, $p = .04$, such that a higher number of pregnancies across the lifespan was associated with poorer sleep quality during the current pregnancy. In addition, prenatal emotion dysregulation was significantly correlated with prenatal sleep quality, $r(84) = -.28$, $p = .01$, such that greater difficulties with regulating emotions were associated with poorer sleep quality.

Emotion dysregulation was also significantly correlated with maternal trauma history, $r(84) = .30$, $p = .01$. Specifically, more traumatic life experiences were associated with higher levels of prenatal emotion dysregulation. Finally, maternal trauma history was significantly correlated with parity, $r(84) = .24$, $p = .03$, such that women who reported more pregnancies over their lifespan also endorsed more traumatic life experiences.

Hierarchical Linear Regression Models (Table3)

In block 1 of the model, results indicated that parity predicted prenatal sleep quality, $B = -.16$, $\beta = -.23$, $p = .04$. For each additional pregnancy in one's life, prenatal sleep quality declined by .16 units. In block 2, emotion dysregulation and maternal trauma history were entered into the model, and the effect of parity on prenatal sleep quality became nonsignificant, $B = -.15$, $\beta = -$
.21, $p = .06$. Instead, maternal emotion dysregulation emerged

as a significant predictor of prenatal sleep quality, B = -.01, β = -.28, p = .01. For every one unit increase in emotion dysregulation, prenatal sleep quality declined by .01 units. Maternal trauma history did not predict prenatal sleep quality, B = .01, β = .03, p = .78. Finally, in block 3, results indicated that there was not a significant interaction between emotion dysregulation and maternal trauma history on prenatal sleep quality, B = .00, β = -.05, p = .68. Overall, this regression model accounted for approximately 13% of the variance in prenatal sleep quality.

DISCUSSION

The overarching aim of this study was to examine how maternal trauma history may be related to sleep quality during pregnancy. We also examined if the relation between maternal trauma history and sleep quality may vary across levels of emotion dysregulation. Results indicated that higher levels of emotion dysregulation predicted poorer sleep quality during pregnancy. However, maternal trauma history did not predict prenatal sleep quality, and this relation did not vary across levels of emotion dysregulation.

Our finding that emotion dysregulation predicted poor prenatal sleep quality is consistent with previous research on psychopathology and sleep health. Pregnancy is a time of significant psychological, social, and neurobiological changes, which oftentimes are associated with stress and risk for psychopathology (Brummelte et al., 2010; Dhillon et al., 2017; Escott et al., 2004; Evans et al., 2001; Furtado et al., 2018; Grote et al., 2010; Heron et al., 2004; Huizink et al., 2004; Lutterodt et al., 2019; Penner & Rutherford, 2022; Rich-Edwards et al., 2006; Wallace & Araji, 2020). Pregnancy-related stressors can prompt emotional, cognitive, and physiological arousal, which reduces

sleep quality (Fairholme & Manber, 2015; Vafapoor et al., 2018; Vandekerckhove & Wang, 2017). Similarly, difficulties with emotion regulation have been found to be associated with depression, borderline personality disorder, anxiety, and self-injurious thoughts and behaviors among pregnant women, and disturbed sleep is a common characteristic of these psychopathologies (Ennis et al., 2017; Lin et al., 2019; Palmer & Alfano, 2017).

Just as the prenatal period may be a time of heightened risk for psychopathology, it also is a time when intervention efforts can have lasting positive effects on maternal postnatal health (Braeken et al., 2016; Duncan & Bardacke, 2009; Dunn et al., 2012; Milgrom et al., 2011; Pat et al., 2019; Spinelli & Endicott, 2003), offspring development (Braeken et al., 2016; Glover, 2014; van den Heuvel et al., 2015), and maternal-infant relationships (de Campora et al., 2014; Duncan & Bardacke, 2009; Glover, 2014; Milgrom et al., 2011). There is a growing body of research demonstrating that mindfulness-based interventions, Dialectical Behavior Therapy-informed skills groups, and mentalization-based treatments can be adapted for pregnant populations and significantly improve emotion regulation skills (Lucena et al., 2020; Penner & Rutherford, 2022; Slade et al., 2019; Wilson & Donachie, 2018). Given our finding that emotion dysregulation predicted poor prenatal sleep quality, it may be that these emotion regulation interventions also could improve perinatal sleep health. Thus, we encourage researchers to include measures of sleep health, including subjective sleep quality, in their studies on perinatal emotion regulation interventions.

Our finding that maternal trauma history did not predict

prenatal sleep quality was unexpected. Although previous studies have reported significant associations between maternal trauma history and sleep (Gelaye et al., 2015; Miller-Graff & Cheng, 2017; Nevarez-Brewster et al., 2022; Sanchez et al., 2016; Takelle et al., 2022), most studies did not include measures of emotion dysregulation. Our results indicated that maternal trauma history was associated with emotion dysregulation, and emotion dysregulation predicted poor sleep quality. It may be worthwhile to examine these relations over time to determine if emotion dysregulation is a mechanism through which maternal trauma history influences perinatal sleep quality. In addition, it may be worthwhile to distinguish between *types* of maternal trauma. Prior research has found that childhood threat experiences, such as physical, sexual, and emotional abuse and exposure to violence, were highly associated with emotion dysregulation and, in turn, mental health problems, but childhood deprivation experiences, such as physical and emotional neglect and separation from primary caregivers, were not associated with emotion dysregulation (Greene et al., 2021). It will be important for future research on maternal trauma history and perinatal sleep to adopt a more nuanced and theoretically-informed conceptualization of traumatic life experiences.

Strengths and Limitations

The present study had several strengths,

including a novel participant recruitment strategy using a measure of emotion dysregulation, which yielded a sample with a wider range of emotional distress. Also, recruitment efforts were made to over-sample for historically minoritized racial and ethnic identities, which increased the diversity of the sample and generalizability of the results. However, there are limitations to address in future research. First, all of the measures were self-reported, which could be prone to social desirability and common- method biases. Second and relatedly, our measure of maternal trauma history was retrospective, which renders it susceptible to recall biases and limited agreement with prospective measures (Baldwin et al., 2019; Newbury et al., 2018; Talari & Goyal, 2020).

However, low agreement between retrospective and prospective measures of childhood maltreatment may not necessarily imply that retrospective measures have poor validity (Baldwin et al., 2019). Additional research has suggested that prospective measures may only account for the most severe childhood maltreatment cases and that retrospective childhood maltreatment measures are more strongly associated with subjective measures of physical and emotional health (Reuben et al., 2016). Thus, children prospectively identified as having experienced maltreatment may have different risk pathways than adults reporting childhood maltreatment retrospectively (Baldwin et

al., 2019). Ultimately, researchers must be aware of these measurement differences and limitations, as they may capture different risk pathways based on their chosen method (Baldwin et al., 2019).

Conclusions and Future Directions

In sum, results from the current study indicate that emotion dysregulation may increase risk for poor sleep quality during pregnancy. Given that prenatal sleep has been associated with maternal postnatal health (Chang et al., 2010; Facco et al., 2017; Okun et al., 2009; Pietikäinen et al., 2018; Sharkey et al., 2016), offspring development (Armstrong et al., 1998; Lahti-Pulkkinen et al., 2018; Li et al., 2023; Micheli et al., 2011; Plancoulaine et al., 2017), and maternal-infant relationships (Chang et al., 2010; Ciciolla et al., 2022; Gordon et al., 2021; Newland et al., 2016; Okun et al., 2009; Pietikäinen et al., 2018; Pires et al., 2010), our study highlights the potential relevance of emotion regulation interventions during pregnancy for improving maternal sleep health. In addition to future studies measuring these constructs over time and distinguishing between types of maternal trauma history, researchers may consider examining bidirectional relations between emotion dysregulation and sleep quality because prior research has indicated that poor sleep quality also can increase emotion dysregulation (Kahn et al., 2013; Palmer & Alfano, 2017; Vandekerckhove & Wang, 2017; Walker & Helm, 2009). The current

study is one of few to examine emotion dysregulation during pregnancy and provides additional evidence that it may be an important factor for identifying mental health concerns during pregnancy. Identifying risk and protective factors for prenatal health is essential to inform intervention efforts for pregnant people and their families.

REFERENCES

Alvarez, G. G., & Ayas, N. T. (2004). The impact of daily sleep duration on health: A review of the literature. *Progress in Cardiovascular Nursing*, *19*(2), 56–59. https://doi.org/10.1111/j.0889-7204.2004.02422.x

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Appleton, A. A., Buka, S. L., McCormick, M. C., Koenen, K. C., Loucks, E. B., Gilman, S. E., & Kubzansky, L. D. (2011). Emotional functioning at age 7 years is associated with C-reactive protein in middle adulthood. *Psychosomatic Medicine*, *73*(4), 295–303. https://doi.org/10.1097/psy.0b013e31821534f6

Armstrong, K. L., O'Donnell, H., McCallum, R., & Dadds, M. (1998). Childhood sleep problems: Association with prenatal factors and maternal distress/depression. *Journal of Paediatrics and Child Health*, *34*(3), 263–266. https://doi.org/10.1046/j.1440-1754.1998.00214.x

Baldwin, J. R., Reuben, A., Newbury, J. B., & Danese, A. (2019). Agreement between prospective and retrospective measures of childhood maltreatment: A systematic review and

meta-analysis. *JAMA Psychiatry, 76*, 584– 593. https://doi.org/ 10.1001/jamapsychiatry.2019.0097

Ball, T. M., Ramsawh, H. J., Campbell-Sills, L., Paulus, M. P., & Stein, M. B. (2012). Prefrontal dysfunction during emotion regulation in generalized anxiety and panic disorders.

Psychological Medicine, 43(7), 1475–1486. https://doi.org/ 10.1017/s0033291712002383

Braeken, M. A. K. A., Jones, A., Otte, R. A., Nyklíček, I., & Van den Bergh, B. R. H. (2016). Potential benefits of mindfulness during pregnancy on maternal autonomic nervous system function and infant development. *Psychophysiology, 54*(2), 279–288. https://doi.org/10.1111/psyp.12782

Brummelte, S., & Galea, L. A. M. (2010). Depression during pregnancy and postpartum: Contribution of stress and ovarian hormones. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 34*(5), 766–776. https://doi.org/10.1016/j.pnpbp.2009.09.006

Buckley, T. M., & Schatzberg, A. F. (2005). On the interactions of the hypothalamic-pituitary- adrenal (HPA) axis and sleep: Normal HPA axis activity and circadian rhythm, exemplary sleep disorders. *The Journal of Clinical Endocrinology & Metabolism, 90*(5), 3106–3114. https://doi.org/10.1210/ jc.2004-1056

Carney, C. E., Buysse, D. J., Ancoli-Israel, S., Edinger, J. D., Krystal, A. D., Lichstein, K. L., & Morin, C. M. (2012). The consensus sleep diary: Standardizing prospective sleep self- monitoring. *Sleep, 35*(2), 287–302. https://doi.org/10.5665/ sleep.1642

Chang, J. J., Pien, G. W., Duntley, S. P., & Macones, G. A. (2010). Sleep deprivation during pregnancy and maternal and fetal outcomes: Is there a relationship? *Sleep Medicine Reviews, 14*(2), 107–114. https://doi.org/10.1016/j.smrv.2009.05.001

Ciciolla, L., Addante, S., Quigley, A., Erato, G., & Fields, K. (2022). Infant sleep and negative reactivity: The role of maternal adversity and perinatal sleep. *Infant Behavior and Development, 66,* 101664. https://doi.org/10.1016/j.infbeh.2021.101664

Clement-Carbonell, V., Portilla-Tamarit, I., Rubio-Aparicio, M., & Madrid-Valero, J. J. (2021). Sleep quality, mental and physical health: A differential relationship. *International Journal of Environmental Research and Public Health, 18*(2), 460. https://doi.org/10.3390/ijerph18020460

Colten, H. R., & Altevogt, B. M. (2006). *Sleep disorders and sleep deprivation an unmet public health problem.* Institute of Medicine.

Crowell, S. E., Puzia, M. E., & Yaptangco, M. (2015). The ontogeny of chronic distress: Emotion dysregulation across the life span and its implications for psychological and physical health. *Current Opinion in Psychology, 3,* 91–99. https://doi.org/10.1016/j.copsyc.2015.03.023

de Campora, G., Giromini, L., Larciprete, G., Li Volsi, V., & Zavattini, G. C. (2014). The impact of maternal overweight and emotion regulation on early eating behaviors. *Eating Behaviors, 15*(3),

403–409. https://doi.org/10.1016/ j.eatbeh.2014.04.013

Dhillon, A., Sparkes, E., & Duarte, R. V. (2017). Mindfulness-based interventions during pregnancy: A systematic review and meta-analysis. *Mindfulness*, *8*(6), 1421–1437. https://doi.org/ 10.1007/s12671-017-0726-x

Duncan, L. G., & Bardacke, N. (2009). Mindfulness-based childbirth and parenting education: Promoting family mindfulness during the perinatal period. *Journal of Child and Family Studies*, *19*(2), 190–202. https://doi.org/10.1007/ s10826-009-9313-7

Dunn, C., Hanieh, E., Roberts, R., & Powrie, R. (2012). Mindful pregnancy and childbirth: Effects of a mindfulness-based intervention on women's psychological distress and well- being in the perinatal period. *Archives of Women's Mental Health*, *15*(2), 139–143. https://doi.org/10.1007/ s00737-012-0264-4

Ennis, C. R., Short, N. A., Moltisanti, A. J., Smith, C. E., Joiner, T. E., & Taylor, J. (2017). Nightmares and nonsuicidal self-injury: The mediating role of emotional dysregulation. *Comprehensive Psychiatry*, *76*, 104–112. https://doi.org/10.1016/ j.comppsych.2017.04.003

Escott, D., Spiby, H., Slade, P., & Fraser, R. B. (2004). The range of coping strategies women use to manage pain and anxiety prior to and during first

experience of labour. *Midwifery*, *20*(2), 144–156. https://doi.org/10.1016/j.midw.2003.11.001

Evans, J., Heron, J., Francomb, H., Oke, S., & Golding, J. (2001). Cohort study of depressed mood during pregnancy and after childbirth. *BMJ*, *323*(7307), 257–260. https://doi.org/10.1136/bmj.323.7307.257

Facco, F. L., Grobman, W. A., Reid, K. J., Parker, C. B., Hunter, S. M., Silver, R. M., Basner, R. C., Saade, G. R., Pien, G. W., Manchanda, S., Louis, J. M., Nhan-Chang, C.-L., Chung, J. H., Wing, D. A., Simhan, H. N., Haas, D. M., Iams, J., Parry, S., & Zee, P. C. (2017). Objectively measured short sleep duration and later sleep midpoint in pregnancy are associated with a higher risk of gestational diabetes. *American Journal of Obstetrics and Gynecology*, *217*(4). https://doi.org/10.1016/j.ajog.2017.05.066

Facco, F. L., Kramer, J., Ho, K. H., Zee, P. C., & Grobman, W. A. (2010). Sleep disturbances in pregnancy. *Obstetrics & Gynecology*, *115*(1), 77–83. https://doi.org/10.1097/aog.0b013e3181c4f8ec

Fairholme, C. P., & Manber, R. (2015). Sleep, emotions, and emotion regulation. *Sleep and Affect*, 45–61. https://doi.org/10.1016/b978-0-12-417188-6.00003-7

Fernández-Alonso, A. M., Trabalón-Pastor, M., Chedraui, P., & Pérez-López, F. R. (2012). Factors related to insomnia and sleepiness in the late third trimester of pregnancy. *Archives of Gynecology and Obstetrics*, *286*(1), 55–61. https://doi.org/10.1007/s00404- 012-2248-z

Folk, J. B., Zeman, J. L., Poon, J. A., & Dallaire, D. H. (2014). A longitudinal examination of emotion regulation: Pathways to anxiety and depressive symptoms in urban minority youth. *Child and Adolescent Mental Health*, *19*(4), 243–250. https://doi.org/10.1111/camh.12058

Furtado, M., Chow, C. H. T., Owais, S., Frey, B. N., & Van Lieshout, R. J. (2018). Risk factors of new onset anxiety and anxiety exacerbation in the perinatal period: A systematic review and meta-analysis. *Journal of Affective Disorders*, *238*, 626–635. https://doi.org/10.1016/j.jad.2018.05.073

Gelaye, B., Kajeepeta, S., Zhong, Q.-Y., Borba, C. P. C., Rondon, M. B., Sánchez, S. E., Henderson, D. C., & Williams, M. A. (2015). Childhood abuse is associated with stress- related sleep disturbance and poor sleep quality in pregnancy. *Sleep Medicine, 16*(10), 1274–1280. https://doi.org/10.1016/j.sleep.2015.07.004

Glover, V. (2014). Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *28*(1), 25–35. https://doi.org/10.1016/j.bpobgyn.2013.08.017

Gordon, L. K., Mason, K. A., Mepham, E., & Sharkey, K. M. (2021). A mixed methods study of perinatal sleep and breastfeeding outcomes in women at risk for postpartum depression. *Sleep Health*, *7*(3), 353–361. https://doi.org/10.1016/j.sleh.2021.01.004

Gratz, K. L., & Roemer, L. (2004). Multidimensional assessment of emotion

regulation and dysregulation: Development, factor structure, and initial validation of the difficulties in emotion regulation scale. *Journal of Psychopathology and Behavioral Assessment*, *26*(1), 41–54. https://doi.org/10.1023/b:joba.0000007455.08539.94

Greene, C. A., McCoach, D. B., Briggs-Gowan, M. J., & Grasso, D. J. (2021). Associations among childhood threat and deprivation experiences, emotion dysregulation, and mental health in pregnant women. *Psychological Trauma: Theory, Research, Practice, and Policy*, *13*(4), 446–456. https://doi.org/10.1037/tra0001013

Grote, N. K., Bridge, J. A., Gavin, A. R., Melville, J. L., Iyengar, S., & Katon, W. J. (2010). A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. *Archives of General Psychiatry*, *67*(10), 1012. https://doi.org/10.1001/archgenpsychiatry.2010.111

Hedman, C., Pohjasvaara, T., Tolonen, U., Suhonen-Malm, A. S., & Myllylä, V. V. (2002). Effects of pregnancy on mothers' sleep. *Sleep Medicine*, *3*(1), 37–42. https://doi.org/10.1016/s1389-9457(01)00130-7

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: Insights from HPA axis studies in humans.

Psychoneuroendocrinology, *33*(6), 693–710. https://doi.org/10.1016/j.psyneuen.2008.03.008

Heleniak, C., Jenness, J. L., Vander Stoep, A., McCauley, E., & McLaughlin, K. A. (2015). Childhood maltreatment exposure and disruptions in emotion regulation: A transdiagnostic pathway to adolescent internalizing and externalizing psychopathology. *Cognitive Therapy and Research*, *40*(3), 394–415. https://doi.org/10.1007/s10608-015-9735-z

Heron, J., O'Connor, T. G., Evans, J., Golding, J., & Glover, V. (2004). The course of anxiety and depression through pregnancy and the postpartum in a community sample. *Journal of Affective Disorders*, *80*(1), 65–73. https://doi.org/10.1016/j.jad.2003.08.004

Hofmann, S. G., Sawyer, A. T., Fang, A., & Asnaani, A. (2012). Emotion dysregulation model of mood and anxiety disorders. *Depression and Anxiety*, *29*(5), 409–416. https://doi.org/10.1002/da.21888

Huizink, A. C., Mulder, E. J. H., Robles de Medina, P. G., Visser, G. H. A., & Buitelaar, J. K. (2004). Is pregnancy anxiety a distinctive syndrome? *Early Human Development*, *79*(2), 81–91. https://doi.org/10.1016/j.earlhumdev.2004.04.014

IBM Corp. (2020). IBM SPSS Statistics for Windows (Version 27.0) [Computer software]. IBM Corp.

Jenness, J. L., Peverill, M., Miller, A. B., Heleniak,

C., Robertson, M. M., Sambrook, K. A., Sheridan, M. A., & McLaughlin, K. A. (2020). Alterations in neural circuits underlying emotion regulation following child maltreatment: A mechanism underlying trauma- related psychopathology. *Psychological Medicine*, *51*(11), 1880–1889. https://doi.org/10.1017/s0033291720000641

Kahn, M., Sheppes, G., & Sadeh, A. (2013). Sleep and emotions: Bidirectional links and underlying mechanisms. *International Journal of Psychophysiology*, *89*(2), 218–228. https://doi.org/10.1016/j.ijpsycho.2013.05.010

Kaliush, P. R., Kerig, P. K., Raby, K. L., Maylott, S. E., Neff, D., Speck, B., Molina, N. C., Pappal, A. E., Parameswaran, U. D., Conradt, E., & Crowell, S. E. (under review). Examining implications of the developmental timing of maternal trauma for prenatal and newborn outcomes.

Kalmbach, D. A., Cheng, P., Ong, J. C., Ciesla, J. A., Kingsberg, S. A., Sangha, R., Swanson, L. M., O'Brien, L. M., Roth, T., & Drake, C. L. (2020). Depression and suicidal ideation in pregnancy: Exploring relationships with insomnia, short sleep, and nocturnal rumination. *Sleep Medicine*, *65*, 62–73. https://doi.org/10.1016/j.sleep.2019.07.010

Kennedy, H. P., Gardiner, A., Gay, C., & Lee, K. A. (2007). Negotiating sleep. *Journal of Perinatal & Neonatal Nursing*, *21*(2), 114–122. https://doi.org/10.1097/01.jpn.0000270628.51122.1d

Kerig, P.K. (2018). Emotion dysregulation and childhood

trauma. In T.P. Beauchaine & S.E. Crowell (Eds.), *The oxford handbook of emotion dysregulation* (pp. 264–282). Oxford Library of Psychology. https://doi.org/10.1093/oxfordhb/9780190689285.013.19

Kiecolt-Glaser, J. K., McGuire, L., Robles, T. F., & Glaser, R. (2002). Emotions, morbidity, and mortality: New perspectives from psychoneuroimmunology. *Annual Review of Psychology*, *53*(1), 83–107. https://doi.org/10.1146/annurev.psych.53.100901.135217

Kizilirmak, A., Timur, S., & Kartal, B. (2012). Insomnia in pregnancy and factors related to insomnia. *The Scientific World Journal*, *2012*, 1–8. https://doi.org/10.1100/2012/197093

Lahti-Pulkkinen, M., Mina, T. H., Riha, R. L., Räikkönen, K., Pesonen, A. K., Drake, A. J., Denison, F. C., & Reynolds, R. M. (2018). Maternal antenatal daytime sleepiness and child neuropsychiatric and neurocognitive development. *Psychological Medicine*, *49*(12), 2081–2090. https://doi.org/10.1017/s003329171800291x

Li, Y.-S., Lee, H.-C., Huang, J.-P., Lin, Y.-Z., Au, H.-K., Lo, Y.-C., Chien, L.-C., Chao, H.-J.,Estinfort, W., & Chen, Y.-H. (2023). Adverse effects of inadequate sleep duration patterns during pregnancy on toddlers suspected developmental delay: A longitudinal study. *Sleep Medicine*, *105*, 68–77. https://doi.org/10.1016/j.sleep.2023.02.022

Lin, B., Kaliush, P. R., Conradt, E., Terrell, S., Neff, D., Allen, A. K., Smid, M. C., Monk, C., & Crowell, S. E. (2019). Intergenerational transmission of emotion dysregulation: Part I. psychopathology, self-injury, and parasympathetic responsivity among pregnant women. *Development and Psychopathology,* *31*(3), 817–831. https://doi.org/10.1017/s0954579419000336

Lucena, L., Frange, C., Pinto, A. C., Andersen, M. L., Tufik, S., & Hachul, H. (2020). Mindfulness interventions during pregnancy: A narrative review. *Journal of Integrative Medicine*, *18*(6), 470–477. https://doi.org/10.1016/j.joim.2020.07.007

Lutterodt, M. C., Kähler, P., Kragstrup, J., Nicolaisdottir, D. R., Siersma, V., & Ertmann, R. K. (2019). Examining to what extent pregnancy-related physical symptoms worry women in the first trimester of pregnancy: A cross-sectional study in general practice. *BJGP Open*, *3*(4). https://doi.org/10.3399/bjgpopen19x101674

Madrid-Valero, J. J., Martínez-Selva, J. M., Ribeiro do Couto, B., Sánchez-Romera, J. F., & Ordoñana, J. R. (2017). Age and gender effects on the prevalence of poor sleep quality in the adult population. *Gaceta Sanitaria*, *31*(1), 18–22. https://doi.org/10.1016/j.gaceta.2016.05.013

Mauss, I. B., Troy, A. S., & LeBourgeois, M. K. (2013). Poorer sleep quality is associated with lower emotion-regulation ability in a laboratory paradigm. *Cognition & Emotion*, *27*(3), 567–576. https://doi.org/10.1080/02699931.2012.727783

Medic, G., Wille, M., & Hemels, M. (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, *9*, 151–161. https://doi.org/10.2147/nss.s134864

Micheli, K., Komninos, I., Bagkeris, E., Roumeliotaki, T., Koutis, A., Kogevinas, M., & Chatzi, L. (2011). Sleep patterns in late pregnancy and risk of preterm birth and fetal growth restriction. *Epidemiology*, *22*(5), 738–744. https://doi.org/10.1097/ede.0b013e31822546fd

Milgrom, J., Schembri, C., Ericksen, J., Ross, J., & Gemmill, A. W. (2011). Towards parenthood: An antenatal intervention to reduce depression, anxiety and parenting difficulties. *Journal of Affective Disorders, 130*(3), 385–394. https://doi.org/10.1016/j.jad.2010.10.045

Miller-Graff, L. E., & Cheng, P. (2017). Consequences of violence across the lifespan: Mental health and sleep quality in pregnant women. *Psychological Trauma: Theory, Research, Practice, and Policy, 9*(5), 587–595. https://doi.org/10.1037/tra0000252

Mindell, J. A., & Jacobson, B. J. (2000). Sleep disturbances during pregnancy. *Journal of Obstetric, Gynecologic & Neonatal Nursing, 29*(6), 590–597. https://doi.org/10.1111/j.1552-6909.2000.tb02072.x

Mindell, J. A., Cook, R. A., & Nikolovski, J. (2015). Sleep patterns and sleep disturbances across pregnancy. *Sleep Medicine, 16*(4), 483–488. https://doi.org/10.1016/j.sleep.2014.12.006

Miranda, R., Tsypes, A., Gallagher, M., & Rajappa, K. (2013). Rumination and hopelessness as mediators of the relation between perceived emotion dysregulation and suicidal ideation. *Cognitive Therapy and Research, 37*(4), 786–795. https://doi.org/10.1007/s10608-013- 9524-5

Narayan, A. J., Rivera, L. M., Bernstein, R. E., Harris, W. W., & Lieberman, A. F. (2018). Positive childhood experiences predict less psychopathology and stress in pregnant women with childhood adversity: A pilot study of the benevolent childhood experiences (bces) scale. *Child Abuse & Neglect, 78*, 19–30. https://doi.org/10.1016/j.chiabu.2017.09.022

Nevarez-Brewster, M., Aran, Ö., Narayan, A. J., Harrall, K. K., Brown, S. M., Hankin, B. L., & Davis, E. P. (2022). Adverse and benevolent childhood experiences predict prenatal sleep quality. *Adversity and Resilience Science.* https://doi.org/10.1007/s42844-022-00070

Newland, R. P., Parade, S. H., Dickstein, S., & Seifer, R. (2016). Goodness of fit between prenatal maternal sleep and infant sleep: Associations with maternal depression and attachment security. *Infant Behavior and Development*, *44*, 179–188. https://doi.org/10.1016/j.infbeh.2016.06.010

Okun, M. L., Hanusa, B. H., Hall, M., & Wisner, K. L. (2009). Sleep complaints in late pregnancy and the recurrence of postpartum depression. *Behavioral Sleep Medicine*, *7*(2), 106–117. https://doi.org/10.1080/15402000902762394

Palmer, C. A., & Alfano, C. A. (2017). Sleep and emotion regulation: An organizing, integrative review. *Sleep Medicine Reviews*, *31*, 6–16. https://doi.org/10.1016/j.smrv.2015.12.006

Pan, W.-L., Chang, C.-W., Chen, S.-M., & Gau, M.-L. (2019). Assessing the effectiveness of mindfulness-based programs on mental health during pregnancy and early motherhood – a randomized control trial. *BMC Pregnancy and Childbirth*, *19*(1). https://doi.org/10.1186/s12884-019-2503-4

Penner, F., & Rutherford, H. J. (2022). Emotion regulation during pregnancy: A call to action for increased research, screening, and intervention. *Archives of Women's Mental Health*, *25*(2), 527–531. https://doi.org/10.1007/s00737-022-01204-0

Pietikäinen, J. T., Polo-Kantola, P., Pölkki, P.,

Saarenpää-Heikkilä, O., Paunio, T., & Paavonen, E. J. (2018). Sleeping problems during pregnancy—a risk factor for postnatal depressiveness. *Archives of Women's Mental Health*, *22*(3), 327–337. https://doi.org/10.1007/s00737-018-0903-5

Pires, G. N., Andersen, M. L., Giovenardi, M., & Tufik, S. (2010). Sleep impairment during pregnancy: Possible implications on mother–infant relationship. *Medical Hypotheses*, *75*(6), 578–582. https://doi.org/10.1016/j.mehy.2010.07.036

Plancoulaine, S., Flori, S., Bat-Pitault, F., Patural, H., Lin, J.-S., & Franco, P. (2017). Sleep trajectories among pregnant women and the impact on outcomes: A population-based cohort study. *Maternal and Child Health Journal*, *21*(5), 1139–1146. https://doi.org/10.1007/s10995-016-2212-9

Qualtrics. https://www.qualtrics.com Redcap Project. http://Project-redcap.org

Reuben, A., Moffitt, T. E., Caspi, A., Belsky, D. W., Harrington, H., Schroeder, F., Hogan, S., Ramrakha, S., Poulton, R., & Danese, A. (2016). Lest we forget: Comparing retrospective and prospective assessments of adverse childhood experiences in the prediction of adult health. *Journal of Child Psychology and Psychiatry*, *57*(10), 1103–1112. https://doi.org/10.1111/jcpp.12621

Rich-Edwards, J. W. (2006). Sociodemographic predictors of antenatal and postpartum depressive symptoms among women in a medical group

practice. *Journal of Epidemiology & Community Health*, *60*(3), 221–227. https://doi.org/10.1136/jech.2005.039370

Sanchez, S. E., Islam, S., Zhong, Q.-Y., Gelaye, B., & Williams, M. A. (2016). Intimate partner violence is associated with stress-related sleep disturbance and poor sleep quality during early pregnancy. *PLOS ONE*, *11*(3). https://doi.org/10.1371/journal.pone.0152199

Sedov, I. D., Cameron, E. E., Madigan, S., & Tomfohr-Madsen, L. M. (2018). Sleep quality during pregnancy: A meta-analysis. *Sleep Medicine Reviews*, *38*, 168–176. https://doi.org/10.1016/j.smrv.2017.06.005

Sharkey, K. M., Boni, G. M., Quattrucci, J. A., Blatch, S., & Carr, S. N. (2016). Women with postpartum weight retention have delayed wake times and decreased sleep efficiency during the perinatal period: A brief report. *Sleep Health*, *2*(3), 225–228. https://doi.org/10.1016/j.sleh.2016.05.002

Slade, A., Holland, M. L., Ordway, M. R., Carlson, E. A., Jeon, S., Close, N., Mayes, L. C., & Sadler, L. S. (2019). *Minding the Baby* ®: Enhancing parental reflective functioning and infant attachment in an attachment-based, interdisciplinary home visiting program. *Development and Psychopathology*, *32*(1), 123–137. https://doi.org/10.1017/s0954579418001463

Spinelli, M. G., & Endicott, J. (2003). Controlled clinical trial of interpersonal psychotherapy versus

parenting education program for depressed pregnant women. *American Journal of Psychiatry*, *160*(3), 555–562. https://doi.org/10.1176/appi.ajp.160.3.555

Takelle, G. M., Muluneh, N. Y., & Biresaw, M. S. (2022). Sleep quality and associated factors among pregnant women attending antenatal care unit at Gondar, Ethiopia: A cross- sectional study. *BMJ Open*, *12*(9). https://doi.org/10.1136/bmjopen-2021-056564

Talari, K., & Goyal, M. (2020). Retrospective studies – Utility and caveats. *Journal of the Royal College of Physicians of Edinburgh*, *50*(4), 398–402. https://doi.org/10.4997/jrcpe.2020.409

Vafapoor, H., Zakiei, A., Hatamian, P., & Bagheri, A. (2018). Correlation of sleep quality with emotional regulation and repetitive negative thoughts: A casual model in pregnant women. *Journal of Kermanshah University of Medical Sciences*, *In Press.* https://doi.org/10.5812/jkums.81747

Vandekerckhove, M., & Wang, Y.-lin. (2017). Emotion, emotion regulation and sleep: An intimate relationship. *AIMS Neuroscience*, *5*(1), 1–22. https://doi.org/10.3934/neuroscience.2018.5.1

van den Heuvel, M. I., Johannes, M. A., Henrichs, J., & Van den Bergh, B. R. H. (2015). Maternal mindfulness during pregnancy and infant socio-emotional development and temperament: The mediating role of maternal anxiety. *Early Human*

Development,   *91*(2),   103–108.   https://doi.org/
10.1016/j.earlhumdev.2014.12.003

Walker, M. P., & van der Helm, E. (2009). Overnight therapy? the role of sleep in emotional brain processing. *Psychological Bulletin*, *135*(5), 731–748. https://doi.org/10.1037/a0016570

Wallace, K., & Araji, S. (2020). An overview of maternal anxiety during pregnancy and the postpartum period. *Journal of Mental Health & Clinical Psychology*, *4*(4), 47–56. https://doi.org/10.29245/2578-2959/2020/4.1221

Wilson, H., & Donachie, A. L. (2018). Evaluating the effectiveness of a dialectical behaviour therapy (DBT) informed programme in a community perinatal team. *Behavioural and Cognitive Psychotherapy*, *46*(5), 541–553. https://doi.org/10.1017/s1352465817000790

World Health Organization. (2019). *International statistical classification of diseases and related health problems* (11th ed.). https://icd.who.int/

Table 3.

Predictors of Prenatal Sleep Quality

	Prenatal Sleep Quality				
Predictors	*B*	*SE*	*β*	*t*	*p*
Block 1					
Age	.00	.02	.02	.18	.86
Parity	-.16	.08	-.23	-2.09	**.04**
Block 2					
Age	-.00	.02	-.01	-.10	.92
Parity	-.15	.08	-.21	-1.92	.06
Maternal Trauma	.01	.02	.03	.28	.78
Emotion Dysregulation	-.01	.00	-.28	-2.58	**.01**
Block 3					
Age	-.00	.02	-.02	-.14	.89
Parity	-.16	.08	-.22	-1.96	.05
Maternal Trauma	.01	.02	.03	.29	.78
Emotion Dysregulation	-.01	.00	-.28	-2.51	**.01**
Maternal Trauma × Emotion Dysregulation	.00	.00	-.05	-.42	.68

Note. Significant associations ($p < .05$) are bolded.

Table 2.

Correlations Among Primary Variables of Interest

Variables	1	2	3	4	5
1. Age	—				
2. Parity	.19	—			
3. Prenatal Sleep Quality	-.02	**-.22**	—		
4. Emotion Dysregulation	-.12	.06	**-.28**	—	
5. Trauma History	-.17	**.24**	-.10	**.30**	—

Note. Significant correlations ($p \leq .05$) are bolded.

$50,000 - $74,999	23 (26.8)
$75,000 - $99,999	14 (16.3)
$100,000 or more	18 (20.9)
Missing or decline to answer	2 (2.3)
Primiparity	
Primiparous	27 (31.4)
Not primiparous	59 (68.6)

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134. **Research Reflection by Marissa Larkin**

Marissa Larkin

Faculty Mentor: Sheila Crowell (Psychology, University of Utah)

My undergraduate research experience was completed in the Child Adaptation and Neurodevelopment (CAN) Lab and was amazing! Assisting in research as an undergraduate allowed me to learn significantly more about the scientific process, helped me decide I wanted to go to graduate school, and ultimately made me fall in love with research. Taking what I had learned about research within my various courses throughout my undergraduate career gave me a glimpse into the world of research, but actually conducting research was a whole new world that I am thrilled I had the chance to experience and continue working with.

About the Author

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135.   **Reconstructing Afromontane Fire Regimes: Exploring the Linkages Between the Askumite Empire and Deforestation**

Corinna A. McMurtrey
and Mitchell Power
(Geography)

Faculty Mentor: Mitchell Power (Geography, University of Utah)

ABSTRACT

Ethiopian Afromontane forests are biodiverse ecosystems with high rates of endemism and have become increasingly

fragmented over the centuries from human-driven deforestation. Consequently, the Ethiopian Highlands have experienced environmental degradation over long time scales from soil erosion, prolonged droughts, and biodiversity loss (Teketay et al., 2010). The Tigray Plateau was once home to the Aksumite Empire, an ancient civilization that rose and fell between c. 50 BCE and 700 CE (Ruiz-Giralt et al., 2021). A favorable climate and advanced agricultural systems allowed for productive yields and substantial food storage to support the Aksumite's growing population (Connah, 2004). While the Aksum Kingdom experienced several prosperous centuries, the civilization rapidly and mysteriously fell around 700 CE. One of the leading hypotheses suggests that overexploitation of the land led to crop failures and a downfall of agricultural productivity (Connah, 2004).

Aiming to expand on this original hypothesis, this research explores paleoecological evidence of deforestation of Afromontane through reconstructing fire regimes from a time period before the rise of the Aksumites and comparing those data to several centuries of fire history during the rise of the Aksumite Empire. The two comparative time slices offer evidence of potential differences and/or similarities in reconstructed fire history. The method of this research

includes charcoal analysis of a sediment core, KV-II, collected from Adigrat, Ethiopia in 2019. Results suggest fires occurred on average once every ~10 years before the arrival of the Aksumite people and fire tended to be less frequent, occurring once every ~50 years during the peak of the empire. The significant decrease in fire frequency during Aksumite times suggests an overall reduction in available biomass, including increased consumption of wood fuel and expansion of

croplands, likely contributing to accelerated deforestation of Afromontane forests.

INTRODUCTION

The Aksumite Empire is an ancient Ethiopian civilization that rose and fell between c. 50 BCE and 700 CE (Ruiz-Giralt et al., 2021). The Aksumites gradually gained wealth and subsequent power through their favorable proximity to major world trade routes running through the Nile River, Red Sea, and Indian Ocean (Connah, 2004). This African civilization was an important player in international politics and one of the first to adopt Christianity. They were the first to mint their own coins, created the Ge'ez written language, hosted several urban centers, and constructed impressive architecture. With an advantageous climate, summer rains brought by the Intertropical Convergence Zone (ITCZ), and advanced agricultural systems (including water- storage dams, ox-drawn ploughs, terracing, and irrigation), the Aksumite Empire enjoyed productive yields allowing for substantial food storage to support their growing population (Connah, 2004).

The Aksumite civilization was situated on the Tigray Plateau of the northern Horn of Africa in modern-day Ethiopia. Extending over ~50,000 kilometers along the Ethiopian Highlands, this plateau is largely formed by the volcanic activity accompanying the East African Rift Zone (Ruiz-Giralt et al., 2021). The resulting irregular landscape ranging from 1,000 to 3,000 meters above sea level makes the area particularly vulnerable to climate and land-use changes (Ruiz-Giralt et al., 2021). Ruiz-Giralt et al. in their 2021 article titled, "Human-

Woodland interactions during the Pre-Aksumite and Aksumite periods in NE Tigray, Ethiopia" notes that these sharp

topographical and altitudinal variations greatly impact environmental conditions, producing distinctive microclimates within short distances (Ruiz-Giralt et al., 2021). This vulnerability contextualizes how land-cover changes may have affected the Aksumite Kingdom, as removing vital forests in the region had the capacity to significantly alter the land that the civilization relied on. In fact, geoarchaeological research has shown that intensified land- use and deforestation of the surrounding Afromontane led to soil erosion after 500 CE (Ruiz- Giralt et al., 2021).

With that said, while the Aksum Kingdom experienced several prosperous centuries, the civilization rapidly and mysteriously fell around 700 CE. The leading hypothesis for this collapse suggests that overexploitation of the land led to crop failures and a downfall of agricultural

productivity (Connah, 2004). Karl Butzer, in his 1981 article titled "Rise and Fall of Axum, Ethiopia: A Geo-Archaeological Interpretation," details the factors that likely contributed to the downfall of the once-prosperous Aksum Kingdom: As the population grew to over ~10,000 people, estimated by Butzer (1981), agricultural demands increased, and the intensified land-use caused significant soil erosion. Combined with seasonal flooding during the rainy months, the land was no longer able to support the Aksumites' agricultural needs (Butzer, 1981). More specifically, land-use practices like deforestation, over-intensive cultivation, and livestock grazing compounded effects of environmental hazards and accelerated soil degradation (Bard et al., 2000).

As these studies have shown, deforestation, among other land-use practices, likely contributed to the erosion of soil and subsequent crop failures for the Aksum people. Even today, the Ethiopian Highlands suffer from soil erosion, extended

droughts, and biodiversity loss due to centuries of deforestation, impacting agriculture and threating the welfare of people, animals,

and other organisms (Teketay et al., 2010). As a result of centuries of vegetation removal, modern analogs of Afromontane ecosystems are rare today on the Tigray Plateau, providing a limited understanding of what these forests looked like before human influence. However, opportunities to understand biodiversity on small-scale modern-day church forests, or sacred forests surrounding churches, monasteries, graveyards, and other sacred sites, provide a window into the species composition of undisturbed Afromontane forests. Church forests in the northern highlands and throughout Ethiopia are protected by the Ethiopian Orthodox Church (Wassie et al., 2010). These biodiverse remnants contribute locally to elevated water tables, reduce surface winds, cooler air temperatures, and support a host of bird species and pollinators that in turn support Ethiopia's agricultural needs (Eshete et al., 2022). Insights into native and endemic species from these island oases can aid in forest restoration and conservation efforts.

Figure 1: Aerial image of Church Forest, Northern Ethiopia (churchforests.org)

Throughout my research, I was particularly interested in ancient anthropogenic deforestation and to what extent, if any, it may have been a catalyst to crop failures and the eventual collapse of the Aksumite Empire by ~700 CE. Therefore, this research paper aims to explore evidence of deforestation by reconstructing fire regimes from before and after the rise of the Aksumite Empire. This was done through charcoal analysis of sediment cores collected from Adigrat, Ethiopia in 2019. The charcoal analysis explores microscopic (>125μm) charred plant remains as a proxy for reconstructing fire regimes.

Indicators of deforestation in sedimentary analysis can include shifts in charcoal and/or pollen morphologies and abundance. Assuming the Aksumite civilization cleared and burned forest to develop agricultural land, charcoal abundance should increase at that time. In the case of transforming forest to farmland, pollen evidence may shift from Afromontane Forest species to increased abundance of agricultural and grassland taxa. Through reconstructing the Afromontane fire regime of the Ethiopian Highlands, I hypothesize that deforestation initiated by the Aksumite people caused a shift in fire frequency. Conversely, an alternative working hypothesis for a change in fire regime is that natural climate variability caused extreme wet or dry periods which impacted the frequency of fire events. Potentially, both natural and human impacts are linked to past changes in fire regime. One avenue forward in this research is to consider multiple working hypotheses, as T.C. Chamberlain (1965) argues that this method allows a deeper understanding of the various working agencies that may have influenced the results of a scientific study (Hillborn et al., 1997).

Terwilliger et al. (2013) emphasizes the importance of paleoecolocical research by describing how paleoenvironmental records have been a key component in understanding the nature of links between past environments and the rise and fall of ancient civilizations, and how such

information can be used to manage consequences of environmental changes today (Terwilliger et al., 2013). By understanding how an environment reacted from specific pressures a past civilization inflicted upon it, we can apply that knowledge to conservation practices today in an effort to prevent historical disasters from repeating.

METHODS

Field

My mentor, Dr. Mitchell Power, completed field work in Adigrat, Tigray, Ethiopia in May 2019 collecting sediment cores from valley floor sediments exposed recently through increased erosion. The site was chosen for its proximity to previously excavated Aksumite archaeological sites, located within ~10-15 km in adjacent valley systems, and previous radiocarbon analysis at the KV-II locality, suggesting the valley floor sediment record spans the past four millennia.

Figure 2: Site Map (green: Afromontane Forest) | Kilat Valley | Core Extraction Site

Study Site in Ethiopia
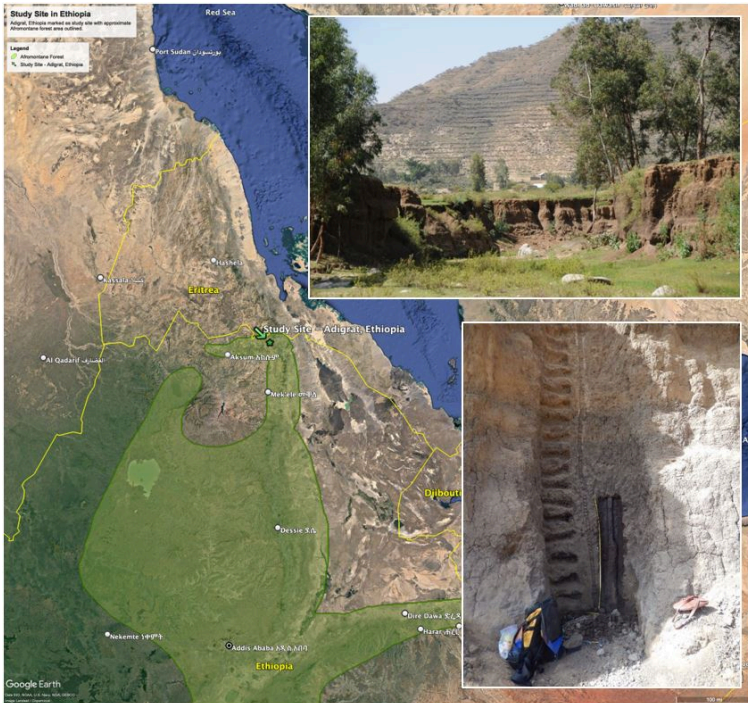
Sediment Core Analysis

To explore evidence of deforestation through charcoal analysis, it was important to examine evidence of fire from before the Aksumite people settled in the region (to use as a control) as well as evidence of fire activity during peak occupation by the Aksumites. Natural disturbance by fire is an essential part of a healthy forest ecosystem, and Afromontane forests likely evolved over thousands of years with lightning-driven wildfires shaping the ecology and natural disturbance regime. The frequency of natural fires is governed by climate, and the climate in Ethiopia is driven by its proximity to the equator, complex topography, and seasonality driven by the ITCZ (Asefa et al., 2020).

Figure 3: Climograph of Adigrat, Ethiopia
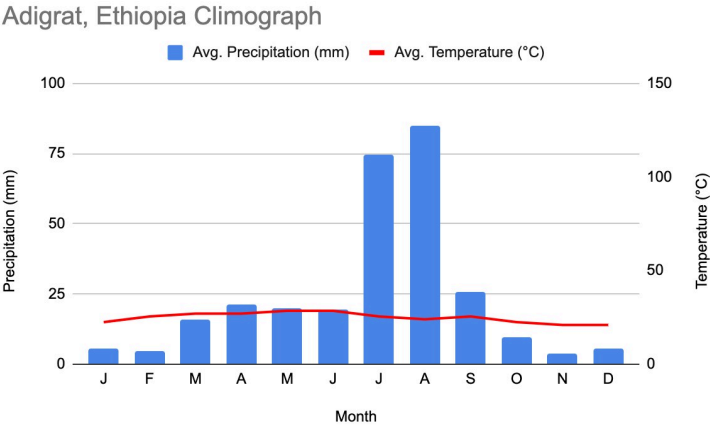
Lab

I began this research journey in the summer of 2022 when we organized the KV-II sediment core segments according to depth and corrected for shrinkage (from water loss while sitting in a fridge for three years) by measuring real vs. original lengths and creating a drawn model of the core. I then created a digital copy of the core using Adobe Illustrator.

Figure 4: Digital and Drawn Stratigraphic Profile of KV-II
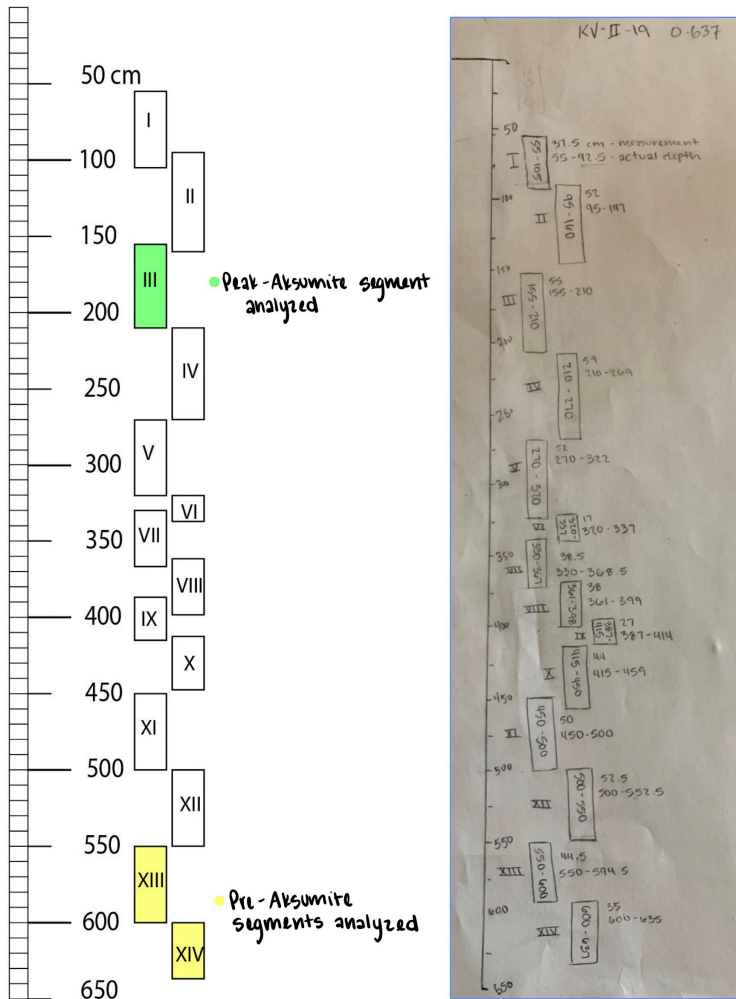
As a chronological control, we selected core segments XIV (depth of 600-637 cm), XIII (550-600 cm), and III (155-210 cm) for sedimentary analysis that would capture pre- and peak-Aksumite occupation. This dating was based on radiocarbon analysis of samples previously submitted by Dr. Power and members of the Ethiopian Tigrai Archaeological Project

(ETAP). Analysis of core segments representing pre- and peak-Aksumite Empire were determined by creating an age model (sediment depth versus time) and choosing sediment depths that aligned most strategically with the timing of occupation.

A total of 230 samples were analyzed in the Power Paleoecology Lab of the Natural History Museum of Utah over the course of two semesters. Sediment processing and analysis took place using the following steps:

- Cut 0.5cm of sediment and bagged in sterile Whirl-Pak bags.

- Subsampled 0.5g for analysis and heated in 5ml of 10% KOH (Potassium Hydroxide).

- Sieve through a 125-micron screen

- Placed sediment residue in labeled petri dishes for microscopic analysis.

- Counted charcoal under microscope at 36X magnification.

Figure 5: Core Segment | Sieved Samples | Microscope | Charcoal Particle
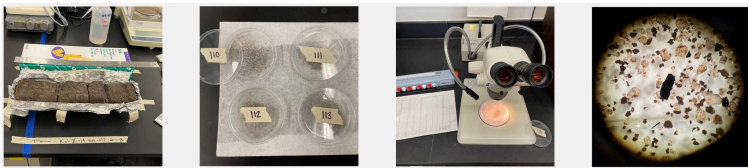
RESULTS & DISCUSSION

Figure 6: Pre-Aksumite Fire History of the Kilat Valley – High Resolution
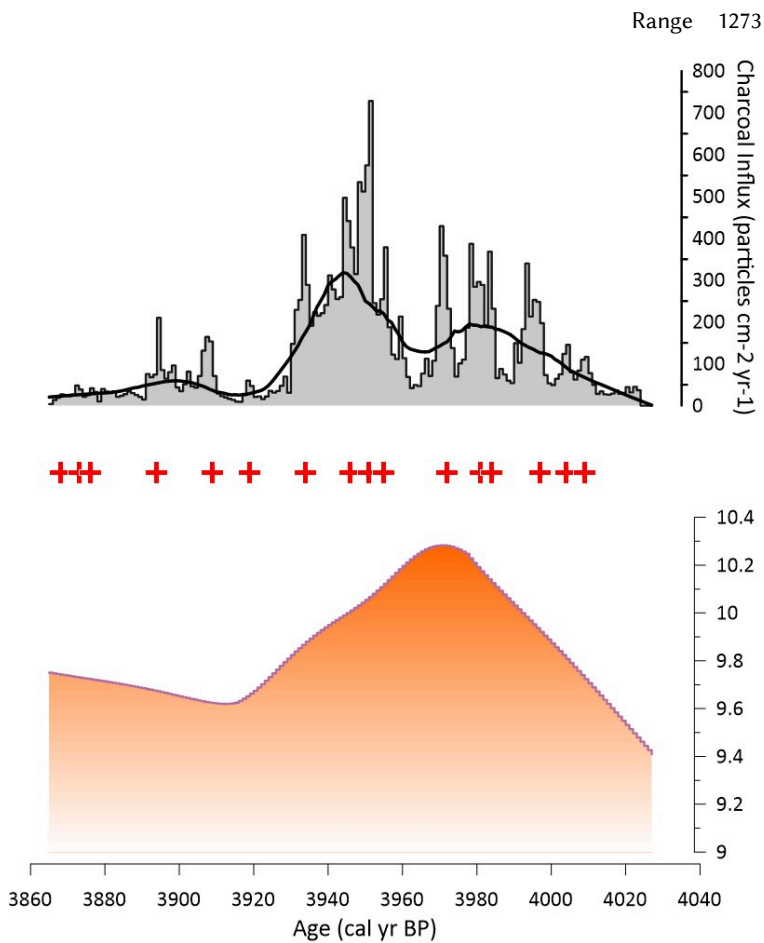
Figure 7: Peak-Aksumite Fire History of the Kilat Valley – High Resolution
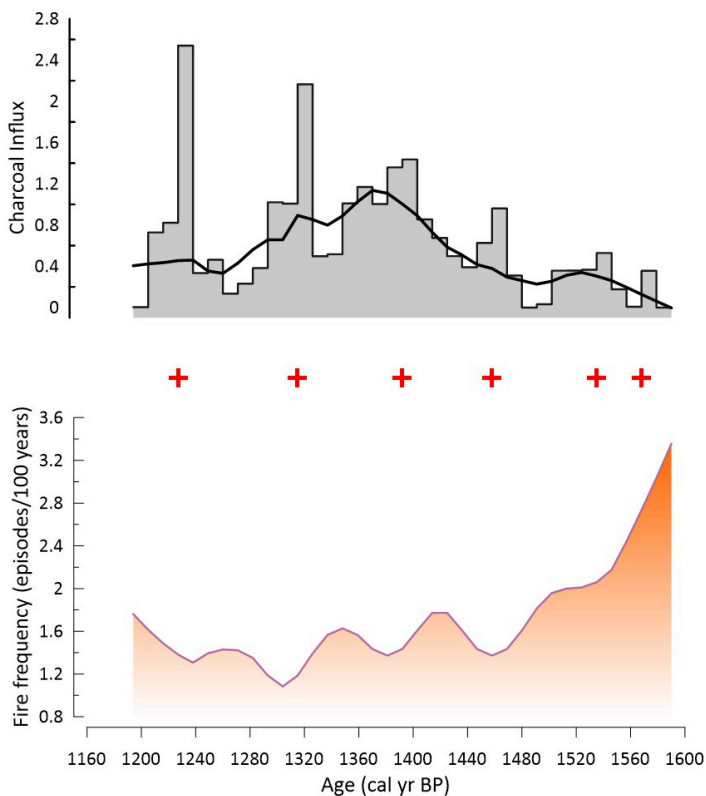
The + symbols in Figures 6 and 7 indicate significant fire events detected with CharAnalysis software (Higuera et al., 2009). In comparing the two time periods of fire history results, the most notable difference is that the average amount of charcoal influx (particles cm-2 yr-1) occur on two different orders of magnitude. For example, the pre-Aksumite fire reconstruction contains influx values exceeding 800 particles cm-2 yr-1, while the peak-Aksumite fire reconstruction includes charcoal influx rarely exceeding 3 particles cm-2 yr-1. Average charcoal influx

between these time periods illustrates the vast difference in fire regimes between pre- and peak-Aksumite times.

Pre-Aksumite Afromontane fire regimes suggest decadal-scale fire events with a total of 16 fires during the ~160 years, prior to Aksumite settlement of the area. Fires of this frequency and magnitude would likely require a healthy forest biomass with sufficient fuels and ignition to produce the high charcoal influx values. The largest fire peak occurred approximately ~3950 yr. BP. Following this significant increase in charcoal, a sharp decline occurred around 3970 yr. BP. This apparent decline in charcoal influx may indicate reduced overall vegetation on the landscape driven by either extreme drought and limited fuel to burn, or conversely, extreme wet conditions with a shortened dry season and extended wet season, which may also explain the decline in fire activity.

Reconstructed fire frequency suggests a higher frequency before the Aksumite Empire settled the area (Figure 6), and lower fire frequency during the peak of the empire's expansion (Figure 7). The Aksumites enjoyed centuries of growth and expansion, with nearly 400 years showing low charcoal influx and only 6 significant fire events, or one occurring every 50 years. The significant decrease in fire frequency points to an overall reduction in fuel availability, potentially aligning with episodes of deforestation, as the Aksumite Empire expanded and citizens consumed wood for fuel, infrastructure, and cooking/heating. The persistent removal of fuel over centuries created a distinct fire regime that suggests humans significantly altered the natural disturbance of Afromontane Forest. Alternatively, the significant decrease in fire frequency during Aksumite times may have been caused by natural climate changes such as a severe dry condition (e.g.

limiting vegetation growth/fuel connectivity) or extreme wet climate conditions (reducing the potential for wildfires to occur and spread). A climate-driven shift in fire regime may be unlikely but could be a contributing factor of the initial expansion and ultimate collapse of the Aksumite Kingdom.

Figure 8: KV-II Radiocarbon Age Model
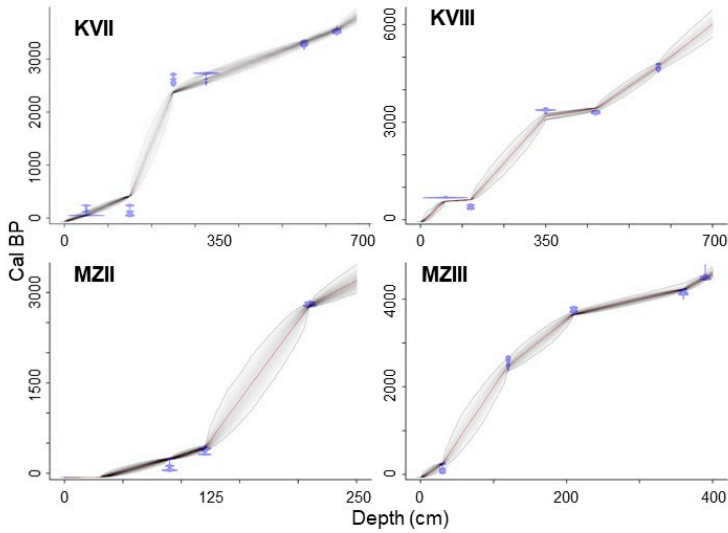
(M. Power, unpublished; Blaauw & Christen, 2011)

A radiocarbon chronology was established through calibrating and modeling the depth:age relationship of six AMS 14C samples (Figure 8). The abrupt change in the age-depth model between 150-250 cm, spans the timeframe of the

Aksumite Empire's peak occupation. The change in sediment deposition rate could be a result of reduced productivity and protracted drought, with a potential link to changes in Afromontane forest abundance.

To understand vegetation changes during the Aksumite Empire, previously published pollen studies offer some insight into vegetation changes. A pollen study conducted on nearby Lake Tana, the largest lake in Ethiopia and the source of the Blue Nile, showed evidence of a severe drought and aridification caused by a southward shift in the ITCZ around 4200 yr. BP. Interestingly, this timeline also coincides with the collapse of Ancient Egypt's Old Kingdom (Marshall et al., 2011). Although the timeline and location differ from the Aksumite Empire, shifts in the ITCZ may have had a similar effect on the Ethiopian Highlands. A second pollen and charcoal study, conducted on two lakes in the Ethiopian Highlands, discovered evidence of vegetation changes over the last 3000 years from anthropogenic impacts, including forest clearing for agricultural land use. As drought caused human populations to relocate, Afromontane forests were able to regenerate (Darbyshire et al., 2003). Few pollen studies have been published from northern Ethiopia, partly because of the limited pollen preservation in the region, highlighting the need for additional studies to understand how the vegetation may have changed temporally and spatially before and during Aksumite times. More palynological studies are needed to provide additional context into the processes involved as well as the timing of forest

loss in the area. Future pollen research should focus on key Afromontane Forest species, including plum pine (*Podocarpus*), juniper (Cupressaceae), olive (*Olea* sp.), and hackberries/nettle trees (*Celtis* sp.).

CONCLUSION

Through this research on changing fire regimes in northern Ethiopia, it is clear that human landcover changes, including the removal of forest for agricultural purposes, began over two millennia ago, and continues today. Afromontane forests of the Ethiopian Highlands, considered one of the world's biodiversity hotspots and hosts over 7,000 plant species, of which over 30% are endemic (CEPF, 2012), have been contracting over time. Therefore, efforts aimed at conservation and restoration of these vulnerable ecosystems (e.g. Asefa et al. 2020) should include information about the long-term role of fire in shaping these ecosystems.

Recent analysis of Afromontane forests in the Horn of Africa (Figure 9) highlights the rich biodiversity and potential threats facing these systems today (Asefa et al., 2020). Presently, crop and livestock agriculture dominate Ethiopia's economy, accounting for 53% of their GDP, supporting 80% of their workforce, and 90% of export earnings (Bishaw, 2001). However, recent and ongoing soil erosion, potentially initiated during the Aksumite Empire, has systematically reduced the amount of soil cover, vegetation and soil moisture retention, and significantly altered the natural fire regime. In a recent study by Bishaw (2001), titled "Deforestation and Land Degradation on the Ethiopian Highlands: A Strategy for Physical Recovery," suggests that modern soil degradation is a direct result of historical and ongoing agricultural practices on the Ethiopian Highlands and is therefore the most immediate

environmental issue facing the country. Given this need for soil conservation in Ethiopia, additional knowledge from land-use activities that may have been linked to the Aksumite Empire's collapse may provide valuable insight for future land management strategies.

Figure 9: Vegetation Schematic (CEPF, 2012; Asefa et al., 2020)

	Vegetation types	Altitude	# of species	Threats
1	Afroalpine & sub-afroalpine	3200–4533 m	22	Agriculture, climate change
2	Dry evergreen montane & grassland complex	1800–3000 m	460	Agriculture, grazing, fuel wood
3	Moist evergreen montane forest	500–2600 m	135	Agriculture, tea & coffee plantations
4	Acacia-Commiphora woodland	900–1900 m	542	Fuel wood, charcoal, agriculture
5	Combretum-Terminalia woodland	500–1900 m	199	Fire, settlement
6	Lowland semi-evergreen forest	450–650 m	101	Fire, infrastructure
7	Desert & semi-desert scrubland	<400 m	131	Invasive species, grazing
8	Aquatic vegetation	Lowlands to highlands	251	Sedimentation, invasive species, change to farmlands

Soil conservation in Ethiopia, including tackling the

challenges of increased soil erosion rates, are directly connected to the conversion of woodlands to croplands (Haregeweyn et al., 2015). Millennia of human activities that have changed forest land and vegetation communities include developing agricultural services and harvesting fuelwood (Teketay et al., 2010). It seems likely deforestation and agricultural practices caused destructive soil erosion during the final days of the Aksumite Empire, as overwhelming evidence suggests these practices and consequences are still occurring today. It's important to note, however, that pollen evidence in sediment core analyses conducted by Darbyshire et al. miraculously signaled that forests in northern Ethiopia could regenerate after as much as 1,800 years of human clearance (Darbyshire et al., 2002). This information is crucial for land conservation in Ethiopia because it indicates that under appropriate land management, with soil and water conservation efforts, forest cover has the capacity to regenerate and mitigate current catastrophes involving soil erosion (Darbyshire et al., 2002).

ACKNOWLEDGEMENTS

training me on various laboratory operating procedures and inspiring me as a fellow woman in STEM. I'm also thankful for my undergraduate research cohort: Talon Roberts and Miranda Clow for shared stories, laughs, and friendship through our combined efforts in paleoecological charcoal analysis in the Power Paleoecology Lab. Thanks to The Natural History Museum of Utah for providing lab space to conduct charcoal analysis; the Office of Undergraduate Research for funding two semesters of my undergraduate research; The Wilkes Center for Climate Science and Policy for funding my graduating semester of research; and the University of Utah Honors College for the opportunity to write this thesis. Finally, I want to extend my deepest gratitude to the Tigrayan people for sharing their land for this research which has truly enriched my personal experience in higher education. Wishing wellbeing, health, and peace to the people of Tigray.

REFERENCES

Asefa, M., Cao, M., He, Y., Mekonnen, E., Song, X., & Yang, J. (2020). Ethiopian vegetation types, climate and topography. *Plant Diversity*, *42*(4), 302–311. https://doi.org/10.1016/j.pld.2020.04.004

Bard, K. A., Coltorti, M., DiBlasi, M. I. C., Dramis, F., & Fattovich, R. (2000). The Environmental History of Tigray (Northern Ethiopia) in the Middle and Late Holocene: A Preliminary Outline. *The African Archaeological Review*, *17*(2), 65–86. https://doi.org/https://www.jstor.org/stable/25130694

Bishaw, B. (2001). Deforestation and Land Degradation in the Ethiopian Highlands: A Strategy for Physical Recovery. *Northeast African Studies*, *8*(1), 7–25. https://doi.org/10.1353/nas.2005.0014

Blaauw, M., & Christen, J. A. (2011). Flexible paleoclimate age-depth models using an autoregressive gamma process. *Bayesian Analysis*, *6*(3), 457–474. https://doi.org/10.1214/ba/1339616472

Butzer, K. W. (1981). Rise and fall of Axum, Ethiopia: A geo-archaeological interpretation.*American Antiquity*, *46*(3), 471–495. https://doi.org/10.2307/280596

CEPF. (2012). *Eastern Afromontane*. Critical Ecosystem Partnership Fund. Retrieved May 2, 2023, from https://www.cepf.net/our-work/biodiversity-hotspots/eastern-afromontane

Connah, G. (2004). A trading metropolis on the Ethiopian Plateau. In *Forgotten africa: An Introduction to Its Archaeology* (pp. 69–75). essay, Taylor & Francis Group.

Darbyshire, I., Lamb, H., & Umer, M. (2003). Forest clearance and regrowth in northern Ethiopia during the last 3000 years. *The Holocene*, *13*(4), 537–546. https://doi.org/10.1191/0959683603hl644rp

Eshete, A. W., Dodds, K., & Lowman, M. (2022, December 22). *Hierotopia: Spirituality and Ecology of Ethiopian Church Forests*. Tree Foundation. Retrieved May 1, 2023, from https://treefoundation.org/2022/12/22/hierotopia-spirituality-and-ecology-of-ethiopian- church-forests/

Haregeweyn, N., Tsunekawa, A., Nyssen, J., Poesen, J., Tsubo, M., Tsegaye Meshesha, D., Schütt, B., Adgo, E., & Tegegne, F. (2015). Soil Erosion and Conservation in Ethiopia. *Progress in Physical Geography: Earth and Environment*, *39*(6), 750–774. https://doi.org/10.1177/0309133315598725

Higuera, P. E., Brubaker, L. B., Anderson, P. M., Hu, F. S., & Brown, T. A. (2009). Vegetation Mediated the Impacts of Postglacial Climate Change on Fire Regimes in the South-Central Brooks Range, Alaska. *Ecological Monographs*, *79*(2), 201–219. https://doi.org/10.1890/07-2019.1

Hilborn, R., Mangel, M., & Chamberlain, T. C. (1997). Appendix: The Method of Multiple Working Hypotheses. In *The Ecological Detective: Confronting Models with Data* (pp. 281–293). essay, Princeton University Press.

Marshall, M. H., Lamb, H. F., Huws, D., Davies, S. J., Bates, R., Bloemendal, J., Boyle, J., Leng, J., Umer, M., & Bryant, C. (2011). Late pleistocene and holocene drought events at Lake Tana, the source of the Blue Nile. *Global and Planetary Change*, *78*, 147–161. https://doi.org/10.1016/j.gloplacha.2011.06.004

Msaky, E. S., Livingstone, D., & Davis, O. K. (2005). Paleolimnological investigations of anthropogenic environmental change in Lake Tanganyika: V. Palynological evidence for deforestation and increased erosion. *Journal of Paleolimnology*, *34*(1), 73–83. https://doi.org/10.1007/s10933-005-2398-0

Ruiz-Giralt, A., Bouchaud, C., Salavert, A., Lancelotti, C., & D'Andrea, A. C. (2021). Human- woodland interactions during the Pre-Aksumite and Aksumite periods in northeastern Tigray, Ethiopia: Insights from the wood charcoal analyses from Mezber and Ona Adi. *Vegetation History and Archaeobotany*, *30*(6), 713–728. https://doi.org/10.1007/s00334-021-00825-2

Teketay, D., Lemenih, M., Bekele, T., Yemshaw, Y., Feleke, S., Tadesse, W., Moges, Y., Hunde, T., & Nigussie, D. (2010). Forest Resources and Challenges of Sustainable Forest Management and Conservation in Ethiopia. In T. Tennigkeit & F. Bongers

(Eds.), *Degraded Forests in Eastern Africa: Management and Restoration* (pp. 19–64). essay, Earthscan.

Terwilliger, V. J., Eshetu, Z., Disnar, J.-R., Jacob, J., Paul Adderley, W., Huang, Y., Alexandre, M., & Fogel, M. L. (2013). Environmental changes and the rise and fall of civilizations in the northern Horn of Africa: An approach combining δd analyses of land-plant derived fatty acids with multiple proxies in soil. *Geochimica Et Cosmochimica Acta*, *111*, 140–

161.   https://doi.org/10.1016/j.gca.2012.10.040

Wassie, A., Sterk, F. J., Teketay, D., & Bongers, F. (2010). Church Forests – Relics of Dry Afromontane Forests of Northern Ethiopia: Opportunities and Challenges for Conservation and Restoration. In F. Bongers & T. Tennigkeit (Eds.), *Degraded forests in Eastern Africa: Management and Restoration* (pp. 123–132). essay, Earthscan.

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136. Developing a Protocol for Detecting Volcanism through Andean Lake Sedimentary Records

Lauren Page; Mitchell Power (Geography); and Susana Velásquez-Franco (Geography)

Faculty Mentor: Mitchell Power (Geography, University of Utah)

Volcanism is likely the main contributor to disturbances

made on the surrounding ecosystems in the Andes. Volcanism can be a powerful disturbance agent, creating a range of changes from blasting impacts, to ash fallout and nutrient enrichment, to high species mortality in ecosystems. In the Colombian Andes volcanism has evolved over the past 5 million years with San Diego Cerro Machin Volcanic Tectonic Providence (SCVPT) as the most active volcanic region, and a large contributor to natural disturbances in northern South America. This research seeks to explore and refine laboratory protocols of detecting volcanism through the analysis of lake sedimentary records using a combination of methods. We carry out elementary analysis via Micro XRF data and smear slide analysis, and Loss Of Ignition (LOI) procedures in SDLEx1 52 cm long record that spans the last 1200 years. Our preliminary analysis shows that Rb, Si, Zr, & K, which are related to volcanic activity of Cerro Bravo and Ruiz volcanoes, spikes around 100 years ago. In smear slide analysis, tephra shards were found throughout several samples, with validation of tephra located in the same core sections analyzed with XRF. Lastly, LOI showed that minerals and organics in the area are strongly correlated and both spike at approximately 0 800 years ago. Combining findings with previous studies can give further analysis of disturbances in Andean lake watersheds. This in turn, can give knowledge on the past frequency and impact of volcanic eruptions to the people living in these watersheds, as we learn more about the ecosystem's responses both locally and regionally.

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137. **Research Reflection by Lauren Page**

Lauren Page

Faculty Mentor: Mitchell Power (Geography, University of Utah)

Before having this opportunity to conduct research, I never foresaw myself going to graduate school. This research has given me a greater understanding of academia and I have learned so much in the past year. I currently am writing this from Colombia, as I am here conducting field research. This trip alone has broadened my world immensely and I know the experiences I have had here will last forever. While the field research itself has been difficult, it has also been rewarding. I can see why people fall in love with research and want to continue and I will say being able to be out in the field after a year in the lab has been incredible. Both Mitch and Susy, my advisors, have been hugely inspirational to me and helped me grow more confident in my abilities to do challenging things.

Whether or not I continue with research and go to graduate school – which I now plan on and hope to do – I know these experiences will be valuable in whatever future of environmental work I pursue.

About the Author

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138. Modern and Prehistoric Geospatial and Climatic Analysis of Aksumite Empire Site on the Tigray Plateau

Talon Roberts and Mitchell Power (Geography)

Faculty Mentor: Mitchell Power (Geography, University of Utah)

ABSTRACT

Ethiopia and the horn of Africa are well known for their vast history of human activity. This history includes some of the oldest hominid remains and ancient civilizations that have come and gone through time, including the Aksumite Empire.

This research strives to better understand the environmental and anthropogenic history of the Aksumite Empire through developing a modern baseline from recent climate and geospatial data in the horn of Africa and comparing that data with long-term paleoclimate archives. This research will explore linkages among climate and fire history from sedimentary charcoal samples collected in 2019 near the city of Adigrat, Ethiopia. Specifically, to investigate what factors may have ultimately led to the collapse of the Aksumite Empire around 700 C.E. A sedimentary archive was used to document environmental changes associated with the development of ancient Aksumite farmlands, where both natural and intentional burning likely occurred. Through examining current climatic data in the horn of Africa and comparing it with long-term paleoclimate reconstructions (e.g., Lamb et al. 2007; Terwilliger et al. 2011) and a newly created sedimentary-charcoal fire history, this research aims to better understand factors that contributed to the decline of the Aksumite Empire. Specifically, this research explores whether natural environmental factors, anthropogenic factors (e.g., over-exploitation of resources), or potentially a combination of several factors contributed to the demise of the Aksumites.

INTRODUCTION

Roughly 2000 years ago, the Aksumite empire was one of the most influential and powerful ancient civilizations of its time. The Aksumites were highly recognized by the likes of the Roman Empire, the Egyptians, and the Greeks, largely due to their high involvement in the trade market and their proximity to the Red Sea (Mokhtar, 1981). This high involvement in the trade market promoted large scale agriculture and material production, and thus rapid advancement and empire expansion. The Aksumite's were also one of the early adopters

of Christianity, which highly influenced their culture and architecture. However, the Aksumite Empire's reign mysteriously ended, and the exact cause for the collapse of the Empire after centuries of success remains a topic of debate. There are many hypotheses that argue the collapse was linked to natural environmental variability in the region, which may have negatively affected their food and supply chains. Other studies have argued that overpopulation mixed with overuse and overreliance on natural resources depleted the land, which could have caused widespread famine (Selassie, 2011) in the region and led to increased tension in the trade market.

Today, people occupy much of the area that was once associated with the Aksumite people, with most of the regional politics during Aksumite times relying heavily on agriculture production to fuel the empire. In this region, natural environmental processes and people's agriculture practices are highly dependent on seasonal variations via the Intertropical Convergence Zone (ITCZ) (Hoegh-Guldberg et al., 2021) and the Indian Ocean Dipole (IOD) (Dubache et al., 2019). The ITCZ is a region near the equator that creates an upwelling effect of moisture from the Indian Ocean. As the Earth's axial tilt changes throughout the year, the ITCZ moves north and south, creating wet and dry seasons. Because of Ethiopia's geographic location residing slightly north of the equator, this region typically experiences one significant rainfall season per year, with the rest of the year receiving minimal precipitation. In addition, because the area relies heavily on the limited wet season, shifts in the climate, including an expansion of the dry season, can vastly affect crop production. With that said,

modern climate change today has already negatively affected this area, as extreme rain and flooding mixed with prolonged, severe drought have dramatically affected people, crop production, and natural ecosystems (Niang et al., 2018).

With such a sensitive ecosystem in place in the Horn of Africa, I hypothesize that drought mixed with overexploitation and overreliance of natural resources led to the rapid decline of the empire. Drought is known to be a large contributor to wildfires, and because there is limited natural vegetation left in the Adigrat area today, I also hypothesize that the intentional burning and land clearing techniques used by the Aksumites could have increased the frequency of wildfires. The role of Aksumites amplifying natural fire regimes coupled with prolonged drought would have further strained the agricultural systems and capacity for people to thrive in this environment. Because the population was large and highly active in the trade market, any large environmental disturbance, such as drought for even a brief period would have severely affected both food production and the overall health of the Aksumites, ultimately leading to the collapse of trade and the Aksumite Empire.

METHODS In The Field

In 2019, Dr. Mitchell Power extracted sediment core samples from a dry riverbed located in the Kilat Valley near Adigrat, Ethiopia. Using the Horn

of Africa as a whole for the geospatial and climatic analysis component of this paper, the Kilat Valley sediment core location on the Tigray Plateau is used to help discern the patterns seen through my geospatial and climatic analysis. It is in these sediment core samples that we can search for evidence of either natural wildfires or intentional agricultural burning through the documentation and frequency of charcoal throughout the sediment core samples.

Figure 1. Study site. The Tigray Plateau is a region of mountainous terrain that is much higher in elevation than the surrounding areas of Ethiopia. The city of Adigrat, as well as the city of Aksum, west of Adigrat and named after the Aksumite people, are both located in this region.

Figure 2. Map of the Horn of Africa in reference to the study site.

Ancient Aksumite Empire Study Location Relative to the Horn of Africa
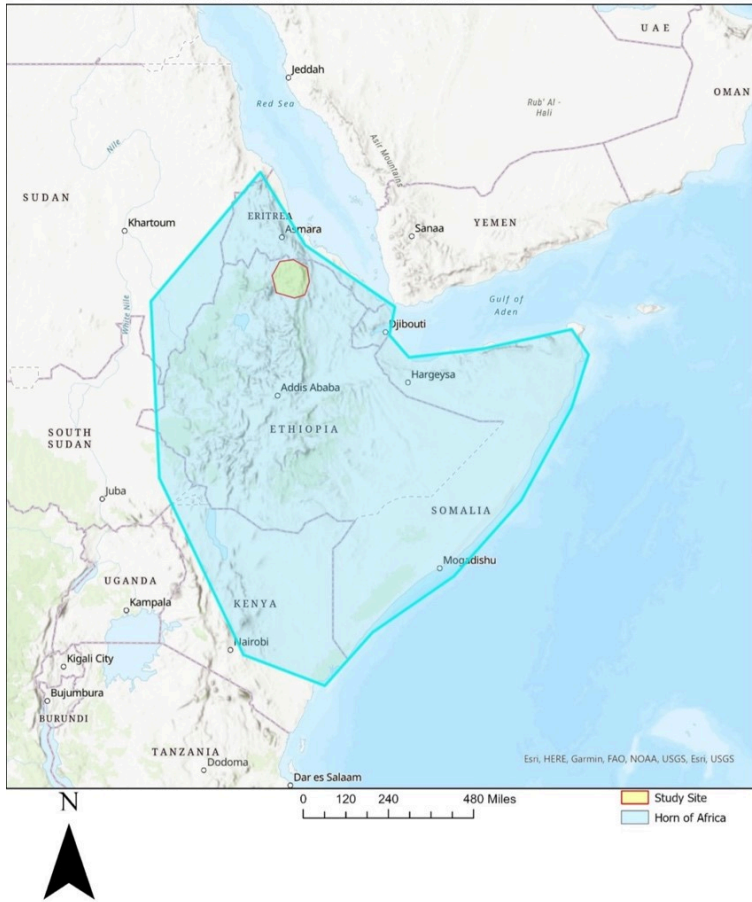
In the Lab

I have tackled this project from two fronts: a historic paleoclimatology approach, and a modern climate analysis approach. For the paleoclimatology approach, I sampled 0.5g of sediment from the sediment cores collected in Adigrat, Ethiopia. I then mixed the samples with

5mL of KOH, sieved them with water at 125 μm, and deposited the sieved samples in petri dishes. Finally, I identified and counted charcoal under a microscope at 3.2X. For the modern climatic analysis, I analyzed and mapped modern climatic data on the Intertropical Convergence Zone (ITCZ) and its effect on the climate of the horn of Africa. Specifically, I wanted to create maps that depict how the ITCZ fluctuates seasonally and affects modern rainfall and vegetation patterns in the Tigray Plateau, Ethiopia.

RESULTS AND DISCUSSION

Figure 3. Charcoal influx (charcoal particles cm-2 yr-1) on the top row, and Fire Frequency (number of discrete charcoal peaks per century) on the bottom row. The left time series represents 1160-1600 cal yr BP, a period of maximum population of the Aksumites) and the right time series represents ~3800-4040 cal yr BP, a time prior to the arrival of Aksumites into the region.
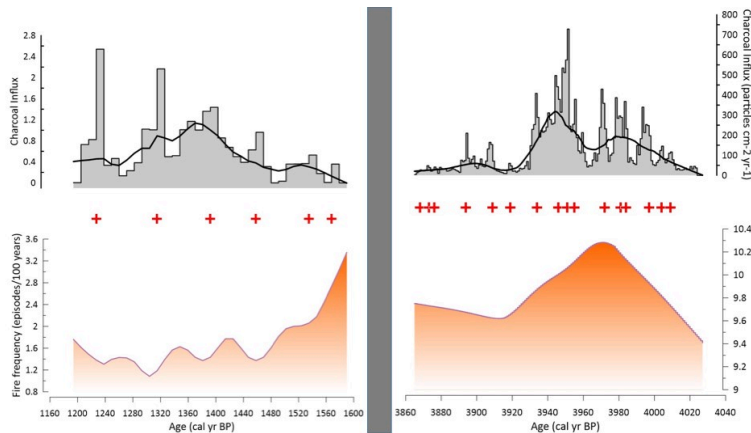
We analyzed three sediment cores for this study. The first

two sediment cores represent data from approximately ~3800-4040 cal yr BP, with this period likely predating notable human settlement and land alteration. With the region of study hosting Afromontane forests, data from our sediment core analysis contained high charcoal influx. This suggests that high magnitude fires were frequent during this time, possibly from natural wildfires in these Afromontane forests. These fires were occurring at about 1 fire every 10 years, with the largest occurring in 2000 BCE (Fig. 3).

The third sediment core represents the time frame of ~1160-1600 cay yr BP, which largely spans the 400-year period that marks the peak of the Aksumite Empire. Through analysis of this sediment core, we found a significant decrease in charcoal influx and about 1/3 as much fire activity compared to previous times. There were 6 notable fire events, and they occurred at an approximate rate of 1 fire every 50 years (Fig. 3). However, further analysis must be conducted to discover what conditions were present during the presumed Empire collapse date of 700 C.E.

Figure 4. Low resolution charcoal influx and fire frequency graph over approximately 3500 years in Adigrat, Ethiopia.
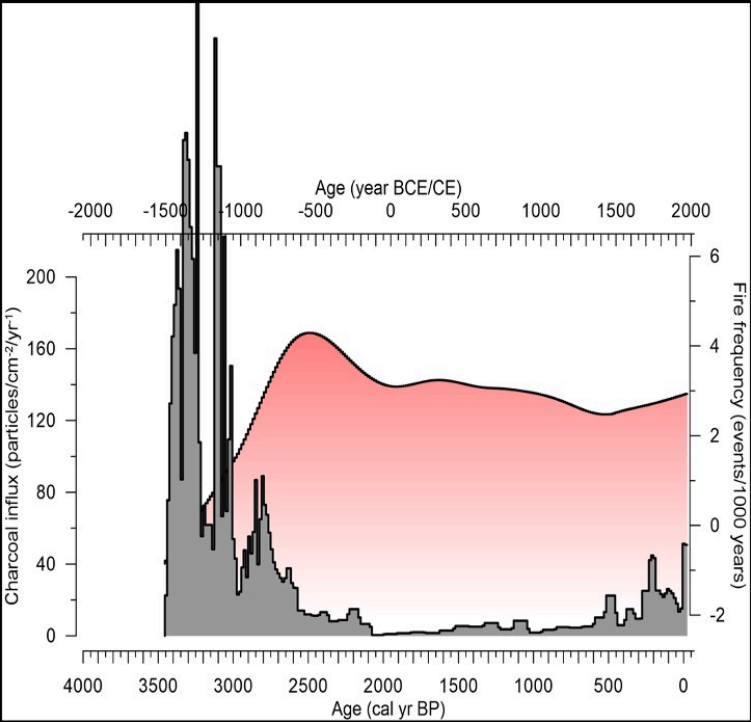
Figure 5. Map of the ITCZ in reference to the Horn of Africa and the study site.
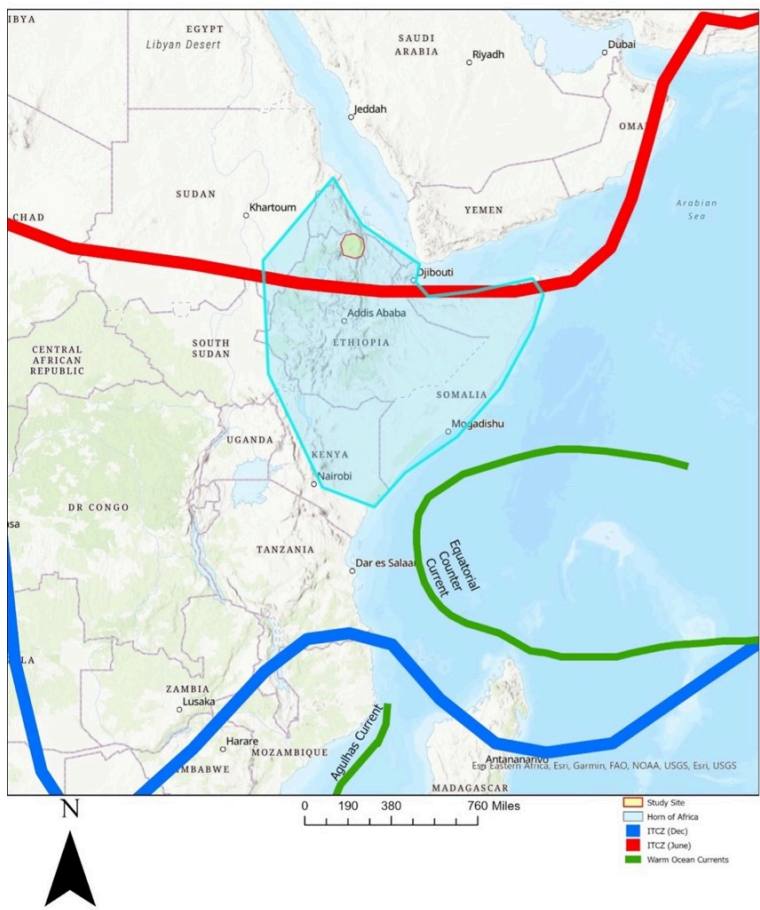
Figure 6. Map of Vegetation Anomalies throughout the Horn of Africa.

April-June 2011 Vegetation Anomalies on the Horn of Africa
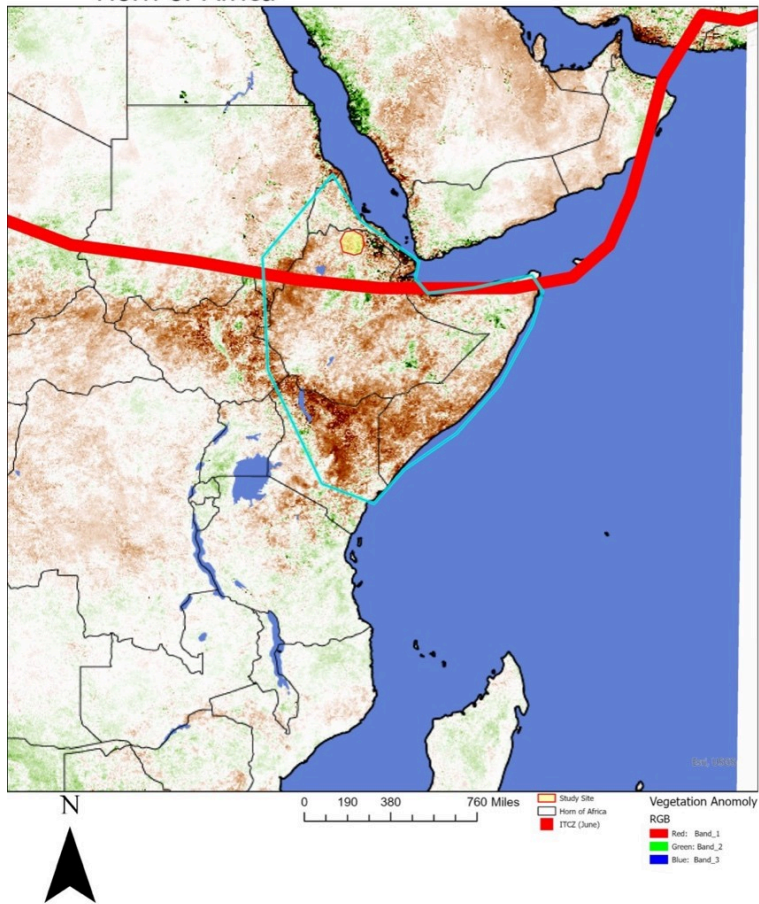
As we can see from the vegetation anomaly (Figure 6) taking place from April-June 2011, the ITCZ is situated at its peak latitude position, leading into what should be the average annual heavy rain season. However, what was supposed to be the start of the rainy season in March and April of 2011 yielded limited precipitation, leading to poor vegetation growth. "The crop grown during this period is typically planted in March or April, when the first rains of the year fall. In 2011, the rains

<u>were late,</u> falling in late April and May, and inadequate (Nasa Earth Observatory, 2011)." Late 2010 also saw little rain in the region.

Figure 7. Rainfall anomalies in the Horn of Africa.

March - May 2017 Rainfall Anomalies on the Horn of Africa
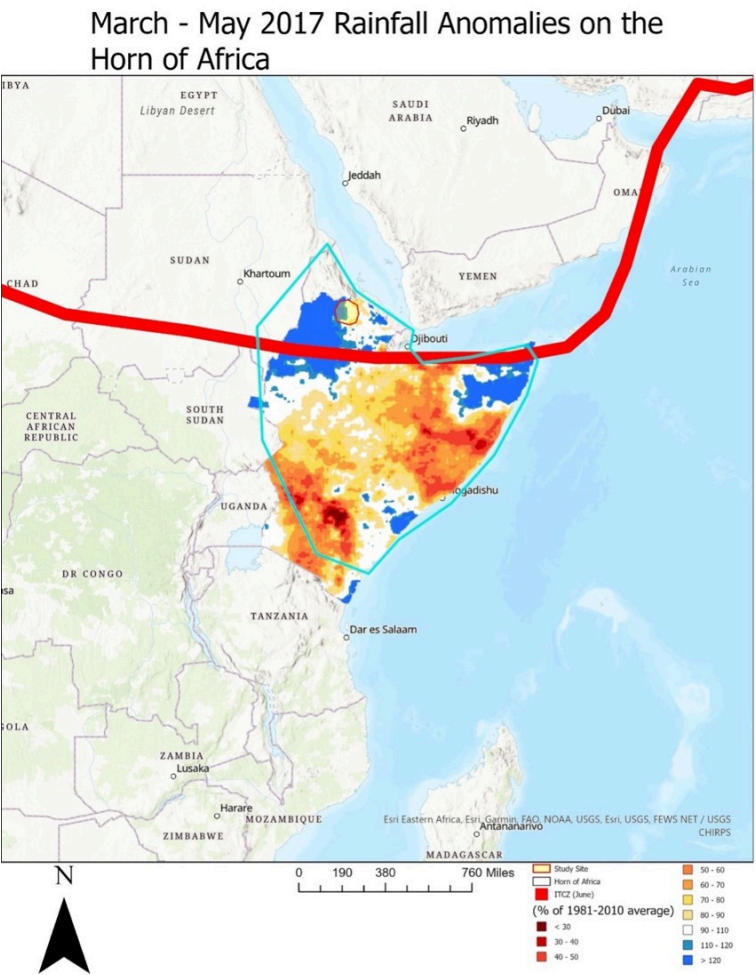
Similarly in 2017, we see more anomalies in Figure 7. However, Figure 7 depicts rainfall

anomalies from March – May 2017, showing where exactly rainfall is occurring in the Horn of Africa. About half of the study site's area was experiencing less than normal rainfall, whereas the rest of the Horn of Africa was experiencing very minimal rainfall. With rainfall being vital to the planting of crops early during the start of the rainy season in March, this lack of rainfall devastated the area. "The strongest El Niño phenomenon on record led to an extreme drought in 2016, with 10.2 million in need of food aid (Anyadike, 2017)."

Figure 8. Map of the ITCZ and its continental effect on rainfall. The Horn of Africa typically receives a small window of opportunity for rainfall. The southwest, central, and southeast portions of Africa receive much more rainfall than the northern section of Africa. This further highlights the importance of an adequate rainy season in the Horn of Africa.
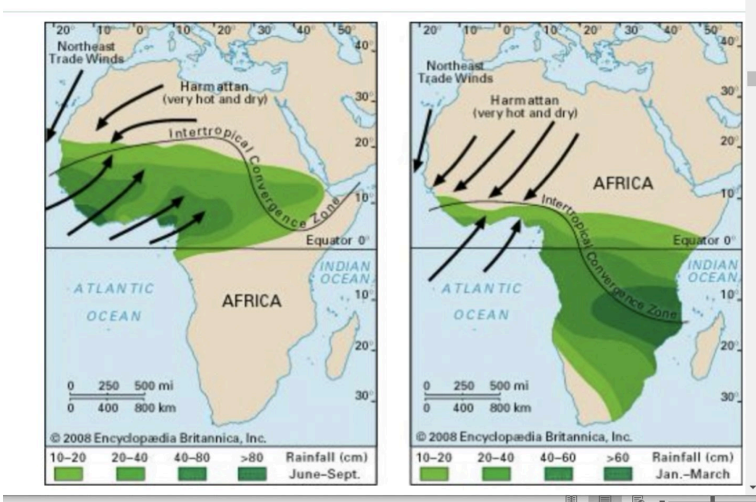
Figure 9. 3-Month Standardized Precipitation Index on the Horn of Africa.
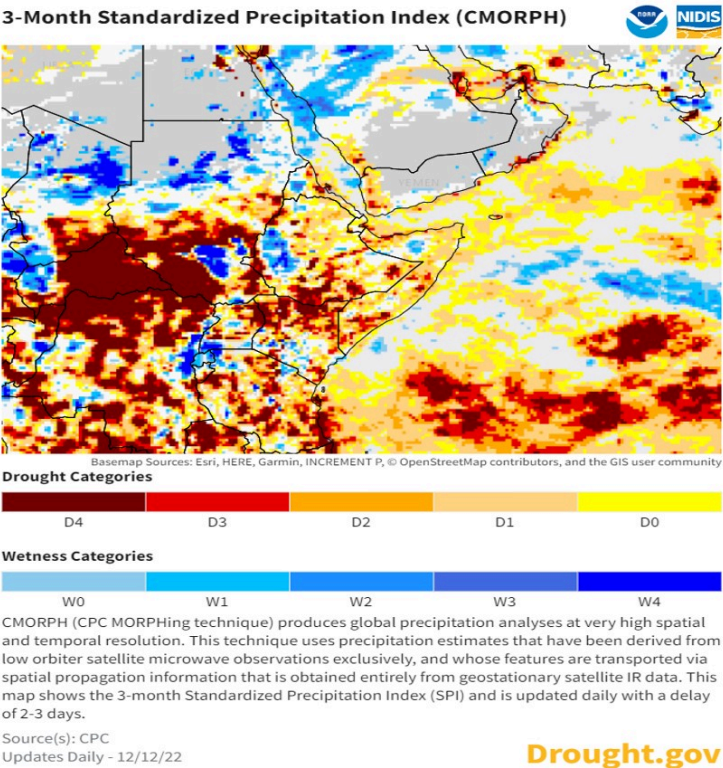
Data from the modern geospatial and climatic analysis of this study shows that drought has been ravaging much of the Horn of African for over a decade, as seen through both rainfall analysis and vegetation anomalies (Figure 6,7, and 9). With climate change undoubtedly underway, changes to the IOD can be a possible contributor to recent ITCZ fluctuations, with La Niña being one of the main drivers for changes in the Indian Ocean Dipole and the ITCZ. "ENSO – La Niña – causes drier than average conditions in the long rainy season (Jimenez, 2021)." Seeing how climatically sensitive this region is, it also supports my research on finding evidence on a possible climate change induced collapse of the Aksumite Empire. Continued

paleoclimatology analysis and research is needed to further support this hypothesis.

However, drought conditions in the Horn of Africa are not new, as the region has been experiencing intense droughts on and off for over 40 years. "Parched ground tells the story of the past 40 years with rainy seasons continually failing in the country (Wentworth, 2022)." Funk et al. also states that, "We present analyses suggesting that warming in the central Indian Ocean disrupts onshore moisture transports, reducing continental rainfall. Thus, late 20th-century anthropogenic Indian Ocean warming has already produced societally dangerous climate change by creating drought and social disruption in some of the world's most fragile food economies (Funk et al., 2008)."

After analyzing how climatically sensitive this region is, fluctuations in the ITCZ and IOD appear to be prominent evidence for explaining a possible climate change induced collapse of the Aksumite Empire. It may be possible that my hypothesis about environmental impacts affecting the Aksumite Empire's collapse, particularly with fluctuations in the IOD and ITCZ, may apply to what is currently occurring in the region today. Facing an array of drought and civil war issues, thousands of people are at risk of starvation, and there have been patterns of drought and flooding that have highly affected the region in the recent past. As stated by the Famine Early Warning Systems Network, "The population in need of humanitarian food assistance in Ethiopia has

reached record levels [1] in 2022 – <u>10 to 15 million people</u> – driven by ongoing insecurity and climate shocks that are likely to result in continued high needs into 2023 (FEWS NET, 2022)."

CONCLUSION AND FUTURE WORK

The prominent role of drought across the Horn of Africa has been linked to natural variation in the ITCZ. Previous work has suggested that changes to the IOD can be one of the biggest drivers impacting the seasonal rainfall associated with the ITCZ. Specifically, during La Niña events, changes in the IOD have been linked to changes in the ITCZ. The role of ENSO variability, particularly during the La Niña phase, causes drier-than-average conditions during the typically lengthy rainy season (Jimenez, 2021). The high climatic sensitivity of this region also supports this research on finding a possible climate change induced cause of the collapse of the Aksumite Empire. A relevant historic climatic event to this study is the Medieval Warm Period event, which occurred within the first few hundred years (~950-1200 CE) after the Aksumite Empire vanished. Changes in charcoal influx are evident during this time, suggesting a potential natural forcing in addition to human-environment changes caused by the Aksumites. Curiously, this also closely coincides with the fall of the Mayan empire at approximately 860 CE, another civilization that may have had a similar relationship with the ITCZ (Brunelle, 2022). Continued paleoclimatology

research and geospatial analysis is needed to further support this hypothesis.

It is still unclear the degree to which fluctuations with the ITCZ and IOD may have contributed to the demise of the Aksumite empire. However, it is clear the ITCZ and IOD play a significant role in the region's climate today. Since agriculture in the Horn of Africa still heavily relies on seasonal rainfall, better understanding past and modern shifts to the ITCZ and IOD may play a vital role for climate change prediction and preparation. By better understanding both past and the present climate data, we can better model and predict how climate may function, change, and affect us in the future.

AKNOWLEDGEMENTS

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REFERENCESNiang, I., O.C. Ruppel, M.A. Abdrabo, A. Essel, C. Lennard, J. Padgham, and P. Urquhart, 2014: Africa. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros, V.R., C.B. Field, D.J. Dokken,

M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L.White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1199-1265.

Hoegh-Guldberg, O., R. Cai, E.S. Poloczanska, P.G. Brewer, S. Sundby, K. Hilmi, V.J. Fabry, and S. Jung, 2014: The Ocean. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros, V.R., C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi,

Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L.White (eds.)].

Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1655-1731.

Dubache, G., Ogwang, B. A., Ongoma, V., & Towfiqul Islam, A. R. M. (2019, December). *The effect of Indian Ocean on Ethiopian seasonal rainfall.* NASA/ADS. Retrieved June 30, 2022, from https://ui.adsabs.harvard.edu/abs/ 2019MAP... 131.1753D/abstract#:~:text=Further%20anal ysis%20reveals%20that%20there,Kenya%2C%20South%20Sud an%2C%20and%20wester n

Terwilliger, V. J., Eshetu, Y. Z., Huang, M., & Alexandre, M. (2010, September 16). *Local variation in climate and land use during the time of the major kingdoms of the Tigray Plateau in Ethiopia and Eritrea.* CATENA. Retrieved June 30, 2022, from https://www.sciencedirect.com/science/article/pii/ S0341816210001232?casa_token=al- fZ5_P1ocAAAAA%3AubSt283gai09V1TWxJu9flPw6Mi23lmNU uFvBHKIMbQ9YD7M _GAiy1dBadlIuMxZ65WYaTw56NW9

Lam, H. F., Leng, M. J., Telford, R. J., Ayenew, T., & Umer, M. (2007, May 1). *Oxygen and carbon isotope composition of authigenic carbonate from an Ethiopian lake: a climate record of the last 2000 years.* The Holocene. Retrieved July 1, 2022, from https://www.researchgate.net/publication/ 37146725_Oxygen_and_carbon_isotope_compos ition_of_authigenic_carbonate_from_an_Ethiopian_lake_A_cli mate_record_of_the_last_2 000_years

Selassie, Y. S. G. (2011). *Plague as a possible factor for the decline and collapse of the ...*

Plague as a Possible Factor for the Decline and Collapse of the Aksumite Empire: a New Interpretation. Retrieved July 1, 2022, from http://www.ityopis.org/Issues- 1_files/ITYOPIS-I-Gebre-Selassi.pdf

Mokhtar , G. (1981). *General history of Africa, II: Ancient civilizations of Africa.*

Unesdoc.unesco.org. Retrieved June 30, 2022, from https://unesdoc.unesco.org/ark:/48223/pf0000184265

Jimenez, Abygail, Kenya Creer, and Valerie Radford. (December, 2021). "A Tale of Two Droughts." https://storymaps.arcgis.com/stories/ c552d8e2e94544b2a607f1fcee749592

NASA. "Severe Drought Causes Famine in East Africa." Earth Observatory. Last modified July 23, 2011. https://earthobservatory.nasa.gov/images/51411/severe- drought-causes famine- in-east-africa.

Wentworth, Adam. "Drought is hitting Ethiopia harder than ever." Climate Home News. Last modified October 27, 2022. https://www.climatechangenews.com/2022/10/27/drought- ishitting-ethiopia-harder-than-ever/

Anyadike, Obi. "Drought in Africa 2017." The New Humanitarian. Last modified March 2017. https://www.thenewhumanitarian.org/feature/2017/03/17/ drought-africa-2017.

Rabarad_FEWSNET. "MAM_Rainfall_Anom." ArcGIS Online. Last modified July 11, 2017. https://www.arcgis.com/ home/item.html?id=841db414a86f40bda7e7da14f759f59e.

NOAA. "National Integrated Drought Information System." Drought.gov/international. Last modified December 14, 2022. https://www.drought.gov/international.

Rahamat, Mohamed Osman. "African Tropical Climates_WFL1." ArcGIS Online. Last modified April 23, 2022 https://www.arcgis.com/home/item.html? id=b4febf65401a4ee5b63a14b41ac95a9f.

Brunelle, A. (Presenter). (2022) Module 16 Lecture

Transcript. Speech presented at the University of Utah, Salt Lake City, UT, Uni

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139. **Fire History and Environmental Disturbance Reconstruction for Fish Lake, UT**

Haley Segura and Andrea Brunelle (Geography)

Faculty Mentor: Andrea Brunelle (Geography, University of Utah)

I am currently working in the Records of Environment and Disturbance (RED) Lab under mentorship of Dr. Andrea Brunelle. I study paleoclimatology by using proxy data, specifically charcoal and pollen from soil cores to aid me in reconstructing the past environment of Fish Lake located in south-central Utah. To determine the age of the core AMS radiocarbon dating was performed to build an age model. Based on the age model, fire history will be reconstructed through

sequential charcoal sampling. Over the past 40 years, the size and duration of wildfires in the western United States have significantly increased (Dennison et al. 2014). Understanding how fire frequency and intensity changes over time is important to those living in the western U.S. Additionally, I will be analyzing 2-3 pollen samples from a particular section of the core and analyzing the paleoecology of Fish Lake at a given point in time to specifically examine the ecological response to a fire.

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140. Research Reflection by Haley Segura

Haley Segura

Faculty Mentor: Andrea Brunelle (Geography, University of Utah)

My undergraduate research experience has been invaluable. I have gained so much more out of my college experience by conducting research. I have gained new insights, experience, and networking that I would not have gained otherwise. I am very grateful to have been given such an amazing opportunity.

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141. **Effect of Covid-19 Pandemic on Oral Health among Uninsured Populations**

Emily Singer and Akiko Kamimura (Sociology)

Faculty Mentor: Akiko Kamimura (Sociology, University of Utah)

Dental services are extremely important to be provided at safety-net health care facilities because uninsured populations tend to otherwise not have access to oral care or education about the importance of maintaining healthy oral care. A major disturbance to many individuals' semi-annual dental checkups occurred with the arrival of the COVID-19 pandemic in 2020. Because the pandemic disproportionately affected minority

populations, a preliminary research hypothesis was that this population's oral health was also disproportionately affected. Along with examining the statistics of how the COVID-19 pandemic has affected underserved, low-income populations, the purpose of this study is to highlight the effects of the pandemic on this population, with a focus on their oral care. Data collection was conducted by distributing surveys to patients of the Maliheh Free Clinic from December 2021 through May 2022 and later analyzed using Statistical Package for the Social Sciences. Research findings reveal that 55.5% of the 254 total study participants needed dental care within the past 12 months but were unable to get treatment, with cost prohibitive reasons representing 60% of people. A study with the clinic conducted prior to the COVID-19 pandemic in 2017 disclosed that 18.3% of the participants received preventive dental care in the past six months (Kamimura, Gull, Weaver, et al., 2017). During the pandemic, 28.7% of research participants visited a dentist in the past six months. Out of this 28.7%, 14.35%, or potentially fewer, visited the dentist seeking preventative care. Time and cost are major obstacles to receiving dental treatment. At the time of the previous study, the clinic provided limited dental care. During the pandemic, the clinic did not provide dental care in person, making it impossible to seek treatment. By comparing the pre-pandemic oral health habits of underserved populations to their current habits as the pandemic continues, my research aids to bridge the unknown gap of the pandemic's true impact on the underserved and ultimately advocates for increased access to dental care at free clinics.

Kamimura, A., Gull, B., Weaver, S., Wright, L., W., Ashby, J., & Erickson, L. (2017). Association Between Health-Related Beliefs

and Oral Health Behaviors Among Uninsured Primary Care Patients. Journal of Primary Care & Community Health. Doi: 10.117/2150131916680887.

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142. **Research Reflection by Emily Singer**

Emily Singer

Faculty Mentor: Akiko Kamimura (Sociology, University of Utah)

Understanding the health of underserved populations is directly related to my future plans as it is my career goal to become a nurse. After earning my Honors Bachelor of Science in Family, Community, and Human Development at the University of Utah, I will attend nursing school to obtain a Bachelor of Science in Nursing. After establishing my career, I hope to travel and provide care to underserved populations to help improve their health. In order to be a well rounded professional in the medical field, I need to learn about how the health of underserved and underrepresented populations is affected by events that affect all populations. Along with the knowledge I gained from the results of my studies, research plays an important role in my educational and career goals.

I learned how conclusions are scientifically drawn, how to support my findings, and how to overcome obstacles as well as enhanced my ability to critically analyze data. Conducting research has prepared me well for the further schooling that lies ahead of me, including nursing school. This specific research project gave me first-hand experience with underserved populations, which will make me better able to serve this population in the future. Recognizing health discrepancies will make me better able to serve my community and ultimately a better nurse.

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143. Parental Involvement in Restorative Justice Programs: Examining Salt Lake Peer Court

Melissa Tyszko

Faculty Mentor: Rebecca Owen (Sociology, University of Utah)

Violence among youth in the United States has been a growing source of concern since the 1990s, when upticks in school-based homicide and mass-shootings led to school boards and government leaders developing stringent discipline policies in education (Rodríguez Ruiz 2017). Most American families felt that these new policies made schools

safer and prevented violent crime, but some students experienced an opposite effect (Rodríguez Ruiz 2017). By the early 2000s, social scientists began questioning the effectiveness of harsh punitive discipline methods, connecting their use to increased youth contact with the juvenile justice system. Especially concerning has been disproportionate juvenile justice contact for disabled youth and youth of color (Hirschfield 2018). These findings led to an investigation of the role of education in youth incarceration, which was soon termed *the school-to-prison pipeline.* Alternative methods of justice have been considered to mitigate the impact of punitive discipline in schools, particularly diversion programs and restorative justice models.

Teen courts quickly became a popular form of diversion to address crime in youth. In Salt Lake City, Utah, a local high school partnered with Salt Lake's Capitol Hill Community Council to establish Salt Lake Peer Court (SLPC) in 1993. The program operates using a restorative justice model, which emphasizes the importance of skill building, accountability, and community connection for at-risk youth (Salt Lake Peer Court 2022). Though diversion programs and restorative justice models have become established as an alternative to the juvenile justice system for youth, research has found mixed results in their ability to prevent youth—especially marginalized youth—from

entering the school-to-prison pipeline, (DeFosset et al. 2017; Kretschmar et al. 2018; Schwalbe et al. 2012). Though previous research on SLPC has found some protective effects of the program for youth of color (Muñoz et al. 2022), there is still much to learn about the effectiveness of the program for youth in Salt Lake.

One previously unstudied aspect of SLPC, which may give insight on the program's success, is the impact of parental involvement. The participation of a parent or legal guardian is required for youth to participate in SLPC, and the quality of parent participation may have an impact on youth success in the program. Research on programs like SLPC shows a promising link between family-involved sentencing and youth success.

Research is difficult to generalize, however, due to variations across program structures and approaches. Family involvement may influence the effectiveness of SLPC for youth participants, but it is necessary to research SLPC individually to determine the role of families on youth success. Using a multi-method design, this study aims to understand the factors which influence parental involvement in SLPC. This was achieved through an analysis of transcripts from previously conducted interviews with SLPC stakeholders, supplemented by observations of SLPC court hearings in the 2022-2023 school year. Factors such as buy-in, parenting style, structural barriers, and

socioeconomic barriers were found to influence parental involvement in SLPC, in part by creating strain which could disincentivize participation. These factors may be considered by SLPC and other youth court programs when developing policies and procedures related to parental involvement.

Parents' involvement in SLPC seemed to be impacted by a combination of factors. It was much more likely for parents with poor participation in the program to have a permissive parenting style, with strong emotional bonds to their child but less consistency in establishing boundaries, supervision, and discipline (Baumrind 2005; Maccoby and Martin 1983). It was also much more common for parents who experienced both structural barriers within SLPC, such as problems with communication and scheduling, and socioeconomic barriers outside of the program, such as a lack of access to transportation, to participate less. Importantly, the combination of a permissive parenting style, structural barriers, and socioeconomic barriers seemed most likely to be associated with poor participation.

The quality of parents' participation had a clear effect on the success of their referred children. At the time of the study, none of the referred youth whose parents had poor participation had graduated. These findings underscore the importance of parent participation in SLPC. Without consistent parent participation, it can be

very difficult to ensure that youth come to return hearings, complete their disposition contracts, and stay in contact with their youth mentors. Many of these cases end up closed, because parents either stop responding to communication or stop attending return hearings. In short, parents are a key aspect of a referred youth's support system; completing the program without them would be incredibly difficult.

The findings from this study seem to suggest three important conclusions; first, poor participation may be associated with parenting style, especially permissive parenting styles. Second, families who experience socioeconomic barriers to participating in SLPC are at greatest risk of having poor parent participation, especially if they also experience structural barriers during their time in the program. Finally, poor parent participation seems to be associated with poor outcomes for referred cases. Therefore, addressing the barriers associated with parenting style and external barriers may improve parent participation, as well as outcomes for referred youth. These findings may have implications on SLPC policies and operations, which could be adjusted to improve accessibility for permissive parents and parents experiencing socioeconomic barriers. Future studies could include interviews with parents to broaden researchers' understanding of their involvement; they could also include a larger

sample size, to represent a broader sample of parenting styles. This could improve overall understandings of the effectiveness of restorative justice and diversion programs like SLPC.

Baumrind, Diana. 2005. "Patterns of Parental Authority and Adolescent Autonomy." *New Directions for Child & Adolescent Development* 2005(108):61–69.

DeFosset, Amelia R., Taylor S. Schooley, Laura S. Abrams, Tony Kuo, and Lauren N. Gase. 2017. "Describing Theoretical Underpinnings in Juvenile Justice Diversion: A Case Study Explicating Teen Court Program Theory to Guide Research and Practice." *Children & Youth Services Review* 73:419–29.

Hirschfield, Paul J. 2018. "The Role of Schools in Sustaining Juvenile Justice System Inequality." *The Future of Children* 28(1):11–35. doi: 10.1353/foc.2018.0001.

Kretschmar, Jeff M., Krystel Tossone, Fredrick Butcher, and Barbara Marsh. 2018. "Examining the Impact of a Juvenile Justice Diversion Program for Youth with Behavioral Health Concerns on Early Adulthood Recidivism." *Children and Youth Services Review* 91:168–76. doi: 10.1016/j.childyouth.2018.06.010.

Maccoby, Eleanor, and John Martin. 1983. "Socialization in the Context of the Family: Parent-Child Interaction." in *Handbook of Child Psychology:*. Vol. 4: Socialization, Personality, and Social Development. New York: Wiley.

Muñoz, Ed A., Rebecca Y. Owen, Moisés Próspero, and Daniel E. Adkins. 2022. "Diversion and Restorative Justice: Salt Lake Peer Court Disrupting Disproportionate Minority Contact?" *Sociology of Race and Ethnicity* 8(2):284–300. doi: 10.1177/23326492221078860.

Rodríguez Ruiz, Rocío. 2017. "School-to-Prison Pipeline: An

Evaluation of Zero Tolerance Policies and Their Alternatives." *Houston Law Review* 54(3):803–37.

Salt Lake Peer Court. 2022. "Salt Lake Peer Court." Retrieved April 25, 2023 (https://www.saltlakepeercourt.org). Schwalbe, Craig S., Robin E. Gearing, Michael J. MacKenzie, Kathryne B. Brewer, and Rawan Ibrahim. 2012. "A Meta- Analysis of Experimental Studies of Diversion Programs for Juvenile Offenders." *Clinical Psychology Review* 32(1):26–33.

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144. **Sleep Quality & Early Life War Exposure: Insomnia Among Vietnamese Older Adults in the Vietnam Health and Aging Study**

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ABSTRACT

We aim to explore the associations between insomnia, early-life war-related stressors, recent life events, and other health and environmental factors in a sample of 2,447 older

Vietnamese adults derived from the 2018 Vietnam Health and Aging Study (VHAS). Insomnia is one of the main symptoms of a variety of adverse health outcomes and sleep disorders but there is a knowledge gap in Low- to Middle-Income Countries (LMICs) like Vietnam. We find that most respondents report moderate to severe insomnia. In ordered logistic regression analyses we find that respondents who served in the military, and who experienced high levels of wartime violence stressors and wartime malevolent conditions, experience more severe insomnia in late adulthood. These associations are mediated by the experience of recent severe PTSD and physical pain. This research makes valuable steps toward understanding war's enduring scars and global efforts to understand, prevent, and treat sleep problems.

INTRODUCTION

"In peace and war, the lack of sleep works like termites in a house: below the surface, gnawing quietly and unseen to produce gradual weakening which can lead to sudden and unexpected collapse."
—Major General Aubrey Newman (Follow Me, 1981, p. 279)

I.   Importance of Healthy Sleep

Sleep is not only comforting and restorative, but essential for our survival. Healthy sleep is characterized by sufficient duration, good quality, appropriate timing and regularity, and the absence of sleep disturbances and disorders (Watson et al., 2015). Since the 1970s, sleep deprivation and disorders have been recognized as a public health concern, but the extent of that concern has emerged mainly in the last two decades (Shepard et al., 2005).

Researchers have associated shorter sleep durations with increased mortality rates, proinflammatory responses, stress responsivity, somatic pain, emotional distress, mood disorders, cognitive deficits, and a reduced quality of life (Kripke et al., 1979; Gildner et al., 2014; Medic et al., 2017). Sleep disorders have also been linked to the development of neurodegenerative disorders like Parkinson's Disease, Alzheimer's Disease, and dementia (Bombois et al., 2010). Important gender distinctions have also been made. Men tend to report higher sleep quality, while women report longer sleep durations (Gildner et al., 2014). Outside of biological and physiological factors, sleep is also "socially driven, dictated by the environment, and subject to interpersonal and societal factors," (Grandner, 2017, p. 1).

II.   Sleep, Military Service & Post-Traumatic Stress Disorder

Data from middle-aged and elderly populations of U.S. veterans indicate that one predictor of sleep problems is exposure to war and combat. In the aftermath of war, veterans experience persistent insomnia and nightmares (Gehrman et al., 2016). This is especially the case when moderated by mental health disorders like PTSD (Armenta et al., 2018). Existing research on U.S. military service members indicates that there is a strong positive association between war exposure and enduring sleep problems. In some studies, up to 90% of Vietnam War veterans experienced post-traumatic stress disorder (PTSD) – 92% of which report experiencing significant insomnia (Gehrman et al., 2016). While the question remains whether one has

a stronger influence on the other, sleep problems relating to PTSD are shown to become independent over time, evolving into a range of other comorbidities (ibid.). Military studies have generally observed that the risk of PTSD is highest in younger age groups; however, when examining persistence, the severity of PTSD has shown to increase with age (Armenta et al., 2018).

III.   Lack of Sleep Research in Low- to Middle- Income Countries

As epidemiological shifts occur worldwide, adequate sleep is becoming more elusive for more people, especially those in lower- to middle-income countries (LMICs). Despite this trend, sleep research has been dominated by and concentrated within high-income populations in temperate climates like the United States and Canada. Most of this research attempts to identify sleep disorders and their correlates, and very few studies attempt to identify associations between early-life war exposure and later-life sleep problems, especially in the developing world (Endshaw, 2012). Inversely, data pertaining to sleep disorders, their relationship to health outcomes, and their potential causes are severely lacking in lower- and middle-income countries (LMICs), especially in rural, tropical regions (Simonelli et al., 2018). The populations in these regions are aging faster than their infrastructures can keep up, which means poor sleep quality and its correlates could present

challenges at the policy, planning, and scientific levels (Simonelli et al., 2018). There are both theoretical and practical gains to be realized by extending research on sleep and sleep disorder to post-conflict and LMICs. Furthermore, as rapid population aging elevates burdens to elder care and healthcare institutions in many LMICs, and Asian LMICs in particular, understanding the roots of insomnia, and undertaking prevention and treatments to address disordered sleeping, can go a long way in supporting older adult health and wellbeing.

What we do know about sleep in LMICs indicates that sleep disorders are prevalent in older populations, especially among older women, and are linked to the same variety of health consequences from studies in high-income countries. In an in-depth, cross-sectional study of 40,000 older adults from eight African and Asian countries, Vietnam stood out as a nation with a high prevalence of sleep problems (Stranges et al., 2012). Overall, 37.6% of women and 28.5% of men reported severe or extreme problems falling asleep, which was second only to Bangladesh (ibid.) These differences could not be entirely explained by poverty, as certain poorer countries such as Tanzania reported a significantly lower prevalence of sleep problems than Vietnam (ibid.). There is currently no research addressing the causality or temporality of sleep disorders in Vietnam or virtually any developing country other than China.

VIETNAM CONTEXT & BACKGROUND

Studying the Vietnamese older adult population provides ample opportunity for examining the link between early-life war exposure and later-life sleep

quality. Vietnam has a long and complex history with war and conflict, but it has achieved a level of stability in the last half-century that allows us to study more direct associations between the American-Vietnam War (1955-1975) and the war cohort's health outcomes.

I.   Before, During, and After the American-Vietnam War

The American-Vietnam War (hereafter "the War") has been described by General Võ Nguyên Giáp as "the most atrocious conflict in human history," (Appy, 2003). When the United States resolved to use force in Vietnam, the North Vietnamese government viewed it as one in a long line of wars fought for Vietnamese independence (Laderman & Martini, 2013). They had already been engaged in centuries of conflict – against the Chinese, Japanese, and most recently, France (ibid.). As the War developed, the United States justified its actions by situating the conflict within the context of the Cold War (ibid.). They were simply there to support the Democratic uprising in the South, led by Ngô Đình Diệm and the successors that took his place after his execution (ibid.). They were not expecting that the guerilla fighters of the North would defeat the United States Armed Forces after twenty years of conflict.

The War was a bloody one, and it led to the death of millions and the destruction of the vital infrastructure   and   landscape   in   Vietnam

(Hirschman, Preston, S., & Loi, 1993). Through relentless bombing campaigns, the United States Air Force destroyed thousands of acres of land in Vietnam and adjacent countries (Miguel & Roland, 2011). This led to the displacement and evacuation of millions of people across the country. Their use of toxic chemicals like Agent Orange caused widespread environmental damage and health problems for not only those who were sprayed, but their posterity too (Leeming, 2015). In an effort to fight back, practically the entire population of Vietnam was involved in one way or another. Men joined military organizations, by choice or compulsion; the North Vietnamese government's propaganda campaign motivated thousands of women and children to join the Youth Shock Brigades; and others still participated in informal military service organizations or helped the war effort as civilians (Guillemot, 2009 & Nguyen, 2017).

Although the North Vietnamese defeated the United States, causing them to retreat from the conflict on April 30, 1975, the War left Vietnam in a state of ruin. The staggering losses experienced by the Vietnamese people had a profound impact on their society, economy, ecology, politics, family structure, and the individual. The concept of post-traumatic stress disorder was not part of the Vietnamese vernacular at the time, but the traumas of war lingered in the soul and psyche of many untreated veterans of the war (Kwon, 2012). Now,

almost fifty years since the end of the war, its assaults on the individual have rarely been studied and its true impact unknown.

II.   The Vietnam Health and Aging Study as a Resource

The Vietnam Health and Aging Study (VHAS) is a key resource for understanding potential associations between war and insomnia severity. It utilizes a life-course conceptual framework and longitudinal design structured to examine relationships between early-life war exposures and later-life health outcomes through a variety of associations. The data in this study has been collected in two waves, the first of which occurred in 2018, the second of which, completed in the summer of 2022, collected follow-up information on participants four years later (Korinek et al., 2019). VHAS thus allows for longitudinal analyses and investigation of late life changes in health and wellbeing. The VHAS has previously identified positive correlations between adolescent war exposure and late-life frailty, cardiovascular diseases, and enduring mental health disorders (Zimmer et al., 2021a; Korinek et al., 2020; Kovnick et al., 2021). Further research is being done to assess the relationships between war and sarcopenia, neurodegenerative diseases, and a range of other health outcomes. Overall, the findings have generally supported a life course theory of health that points to long-term effects of war on health

and aging (Zimmer et al., 2021b). Further research within Vietnam and among its older adult population makes possible a deeper understanding of the relationship between war and sleep, and the mediating factors underlying these associations, such as PTSD, socioeconomic conditions, recent stress events, and proximate health conditions.

HYPOTHESES

Based upon past research and life course approaches to health, namely the cumulative disadvantage theory (e.g., Ferraro et al. 2009), we formulate the following hypotheses:

1) Older adults who served in the military during their young adulthood are more likely to experience severe insomnia in late adulthood as compared to nonveterans.

2) Insomnia severity will be greater among those older adults who encountered relatively numerous and severe exposures to wartime combat and death, and malevolent environmental conditions of war.

3) Due to their influence upon more proximate risks for insomnia, namely PTSD, pain and recent life event stressors, we hypothesize that the hypothesized associations between military service and war-time stressors will be largely attenuated when these current health and stress conditions are incorporated into our models.

By analyzing a population with highly heterogeneous exposures to diverse war-related stressors, we gain unique insights into the role of life course stress as a risk factor for disordered sleep in late adulthood.

RESEARCH METHODS

I.   Study Design and Participants

To estimate the correlates of insomnia severity among Vietnamese older adults we analyse data from the 2018 Vietnam Health and Aging Study (VHAS). The VHAS conducted face-to-face survey interviews and biomarker data collection from May to August 2018 with 2,447 Vietnamese adults aged 60 and older (median year of birth, 1950). VHAS study participants reside in four districts of northern and central Vietnam purposively selected to capture geographic diversity in war exposure as indicated by the intensity of bombings across the Democratic Republic of Vietnam during the 1960s and 1970s (see Figure 1; Miguel & Roland, 2011).Within these four districts, 12 communes were randomly selected for data collection, and stratified random sampling methods, with strata delineating gender and military participation, were implemented to select 204 individuals from each of 12 communes (Korinek et al., 2019). Due to the passage of time between the war's end and VHAS data collection, the sample is selective of those who survived the war, remained in Vietnam, and did not die from other causes prior to 2018. All study participants provided written informed consent to participate, and VHAS received IRB approvals from the University of Utah and Hanoi Medical University. A more detailed description of the VHAS study design and sampling procedures are elaborated elsewhere (ibid.).

II.   Variable Measurement

With an in-depth omnibus survey instrument implemented by Vietnamese interviewers in the homes   of   respondents   (see https://vhas.utah.edu/_resources/documents/vhas-questionnaire- english.pdf), the VHAS provides

detailed data on respondents' life history experiences and exposures during wartime, their family background and migration history, as well as a breadth of information on current health conditions and health behavior, income and assets, household composition and family relationships, and social connections and support. The VHAS is unique in its combination of early life war exposure and late life health data for leveraging analyses of armed conflict's lasting consequences for health and aging.

i.   *Dependent Variable*

We assess our dependent variable, **insomnia severity**, using an ordered categorical variable. The variable is based upon participants' self-reported experience of insomnia in the past month, characterized as none, moderate, or severe.

ii.   *Independent Variables*

We construct four sets of independent variables in order to examine the extent and pathways through which early life experiences of war impact upon insomnia in late life. These variables capture early life stressors linked to one's social positioning in the war, as well as more proximate stress and health conditions known to exacerbate sleep problems. Our first measure of wartime stress exposure is based upon respondents' **military service**, in particular whether they served in the formal military (e.g., the People's Army of Vietnam), an informal military organization (e.g., a paramilitary/militia unit such as the Youth Shock Brigades), or did not complete any military service. We round out our assessment of wartime stressors with two standardized indices assessing respondents' exposure to a series of **war-related**

combat and violence exposures (e.g., their own injury in wartime, having seen dead Vietnamese or foreign soldiers), and their experience of a series of **malevolent environmental conditions during war** (e.g., having to flee due to bombing, severe wartime shortages of food or water, the persistent fear of death, and exposure to toxic chemicals like Agent Orange). Details on the methods and component items utilized to construct the standardized war exposure indices are reported elsewhere (Young et al. 2021).

Next, in light of past research highlighting associations among trauma, PTSD, and insomnia, we employ a categorical variable capturing **recent PTSD symptom severity** reported by VHAS respondents. The VHAS includes nine questions drawn from the 20-item PTSD Checklist (PCL-5), covering each of the four diagnostic clusters for PTSD—reexperiencing, avoidance, dysphoria, and hyperarousal (Price et al. 2016). These items query whether, in the last month, the respondent had experienced (1) repeated, disturbing, and unwanted memories of a stressful wartime experience; (2) strong physical reactions when something reminds them of the experience; (3) avoidance of memories, thoughts, or feelings related to the experience; (4) strong negative beliefs about themselves, other people, or the world; (5) loss of interest in activities they used to enjoy; (6) irritable behavior, angry outbursts, or aggression; (7) feeling jumpy or easily startled; (8) difficulty concentrating; (9) or trouble falling or staying asleep. Items were scored from 0 to 3, capturing whether respondents had experienced a symptom and whether it bothered them *not at all*, *a little bit*, *moderately*, or *a lot*. We removed the final item, trouble falling/staying asleep, from the calculation

of PTSD symptom severity due to the close association (r=0.364) with our dependent variable. We generate PTSD symptom levels – None, A few symptoms, Subclinical, or provisional PTSD — based upon percentage cut-points specified by the VA for the 20-item (48 point) PCL and modified for use with our 8-item (24 point) index. Our cut-point threshold for provisional PTSD (>=10) also considers the underreporting of mental health conditions in Vietnamese culture (for more on this see: Kovnick, et al. 2021; Nguyen 2015; van der Ham et al. 2011; Vuong et al. 2011).

We also incorporate into the models a set of recent stressful life events that we hypothesize will exacerbate insomnia. These indices, which we have standardized on a 0-3 point scale, comprise **family-related stressors, recent personal event stressors, and financial stressors.** Some of the stressors included in the indices are based on questions about martial disruptions, recent deaths in the family, illnesses experienced by family members, accidents that caused physical or psychological harm, major residential moves, and great financial difficulties.

As indicators of **health conditions** which pertain to insomnia severity we include a categorical variable indicating whether the respondent indicates having **experienced bodily pain**, and the degree of pain (whether slight, moderate or severe). **Physical exercise** is also

assessed categorically, and is coded as having participated in no physical exercise in the past year, infrequent physical exercise (monthly-less than weekly), or frequent physical activity (daily or almost daily). As an additional marker of health, we have assessed respondents' **level of frailty**, assessed categorically as "not frail," "very mildly frail," "mildly frail," and "moderately/severely frail."

iii.   Control Variables

Finally, our models incorporate a series of socioeconomic and demographic control variables, namely respondent's age, sex, district of residence, current marital status, current working status, and educational attainment.

III.   Analytical approach

In addition to univariate and bivariate statistics, we estimate nested survey-adjusted generalized ordered logit models in order to examine the associations between variables. We utilize the generalized ordered logit estimation (gologit2) due to a violation of the proportional odds assumption observed for the full model when utilizing ordered logit (ologit) estimation. Through a process of listwise deletion we arrive at an analytical sample size of 2,327. We use Stata 16.1 for all statistical analyses.

RESULTS

I.   Sample Descriptive Statistics

Table 1 provides descriptive statistics for our full analytical sample, as well as for subsamples of men

and women. Descriptive data are unweighted and are presented as percentages or mean values, as appropriate. Our descriptive results demonstrate that insomnia, including severe insomnia, is relatively common within the VHAS sample.

Moreover, women exhibit a greater prevalence of both moderate and severe insomnia compared to men. With respect to our independent variables, high levels of lifetime military service, with approximately two-thirds of the sample indicating either formal or informal service, are in part reflective of military mobilization in the DRV during respondents' youth, but more so a reflection of VHAS subdomain sampling in military service and gender categories. That said, over three-quarters of men in the VHAS sample served in the formal military. A significant minority of women in the sample also served in formal military roles (7.79%), while nearly 40% served in informal military roles, largely as volunteers in the Youth Shock Brigades.

Wartime violence exposures are assessed by standardized indices, centered around zero, thus challenging univariate description. We do observe that men's mean violence exposure index is significantly greater than that of women, while the gender difference in malevolent conditions exposure is statistically insignificant. Likely a reflection of their different military service roles and distinctive degrees of violent stress exposures

in wartime, men and women diverge in their levels of recent PTSD symptoms. Specifically, women are more likely than men to indicate experiencing no recent PTSD symptoms, while men exhibit higher symptom severity consistent with subclinical PTSD and PTSD (provisional).

Life event stressors are also standardized indices which suggest that financial stress is the most prevalent stressor in the VHAS sample, followed by family stressors and personal event stressors. Women report slightly greater levels of family stress than men, and levels of personal event and financial stress that are significantly greater than men. With respect to late life health conditions, we see that significant shares of the VHAS sample suffer from bodily pain, including severe pain (17.88%), with reported degrees of pain greater among women than men. Physical exercise frequency exhibits a bimodal pattern, with a sizable share (45.6%) indicating having done no/almost no physical activity in the past year, and a nearly parallel share (42.11%) indicate having done physical exercise daily/almost daily. Men in the VHAS sample are significantly more likely than women to report daily/almost daily physical activity. We have also observed that about half (50%) of our sample experiences some level of frailty, and roughly 27% experience moderate to severe frailty. This quality is overrepresented in

women, and 32% of the women in this sample experience moderate to severe frailty.

Rounding out our descriptive statistics, we see that nearly three-quarters of the analytical sample is currently married, but women are far less likely to be currently married. Less than 15% completed high school education, and a significantly smaller share of women (8.98%) have this education credential. Many in the sample remain economically active, despite their older ages. Specifically, nearly one half of the sample reports they are currently working for pay/profit.

II.   Bivariate Analyses

We display select bivariate analyses in Figures 4-8, depicting bivariate associations between insomnia and war stress exposures, PTSD Symptom severity, recent life event stressors, pain and frailty level, respectively. Across each of these independent variables, consistent with hypothesized associations, we observe a positive association with insomnia severity. Those with more severe stress exposure during war, worse PTSD, more frequent and severe recent life stressors, worse pain and more frailty exhibit more severe insomnia.

III.   MultivariateModelResults

We report the results of three sets of generalized ordered logit regression results and standard errors in Tables 2a and 2b. Table 2a includes relative odds ratios (exponentiated logit coefficients) for

moderate and severe insomnia combined, with no insomnia as the reference category. Table 2b provides relative odds ratios (exponentiated logit coefficients) for severe insomnia, with reference to moderate and no insomnia combined. In both tables, Model 1 includes military service and a series of covariates. Model 2 incorporates our two war-related stressor indices. Model 3 adds recent life event stressors and health conditions pertinent to insomnia, in particular PTSD and reported regular suffering from physical pain. Because the direction and magnitude of results are largely consistent across Tables 2a and 2b, we concentrate our discussion upon the results, as they relate to our study hypotheses, contained in Table 2a.

The multivariate results in Table 2a are largely consistent with our hypotheses concerning war-related stressors in early life and late life insomnia severity. First, consistent with our first two hypotheses, the generalized ordered logit regression results indicate that war stress exposures, including both military service and indices of violence and malevolent conditions in wartime, each heighten the risk of insomnia among Vietnamese older adults in the analytical sample. However, consistent with our third hypothesis, these war stress exposures are largely attenuated when we incorporate recent PTSD symptom severity into the models.

Older adults' recent experiences of life event stressors, especially family-related stressors (e.g., divorce, the death of a child) and financial stressors, result in heightened risk of insomnia relative to those with fewer/less severe stressful life events. And our hypothesized associations between recent

health conditions and insomnia are partially supported in Model 3. Specifically, older adults reporting moderate and severe bodily pain are significantly more likely to experience insomnia than their pain-free counterparts. We observe no significant association between recent physical activity frequency and insomnia.

In sum, war exposures experienced decades prior, in early adulthood, are relevant to the experience of insomnia among Vietnamese older adults who experienced wide-ranging and diverse wartime stressors in their youth. However, the attenuation of war stress exposure coefficients to statistical insignificance in the second and final model suggests mediating pathways between war stress exposure and insomnia via more proximate health conditions and stressors. Moreover, those who continue to suffer from symptoms of PTSD linked to the hardships and stressors of war are at particular risk for insomnia.

DISCUSSION

The enduring toll of war can be observed far past its immediate casualties, in the morbidities and causes of death among those most negatively impacted by wartime violence. The physical and mental health of those who have been exposed to war can persist for decades. Sleep disorder is a critical domain within which the lasting traumas of war may be apparent, and where war's stressors may deliver ongoing assaults to late life physical health and cognitive functioning. This study extends and supplements this research by finding significant correlations between military service, wartime exposures and later-life insomnia. Through this examination, we are able to foster a greater understanding of the temporality of war and its long-term impact on the sleep quality of the Vietnamese war era cohort.

Our results are suggestive of processes extending from early life stressors in wartime to poorer sleep, as assessed by recent insomnia. Importantly, the experience of proximate physical and mental health conditions appear as key mediators between early life stress in war and late life insomnia. These mediating pathways are suggestive of treatments, such as pain relief, strength training, and mental health counseling, that may alleviate the long-term scars of war and thereby aid in improving sleep quality in those who suffered the greatest levels of stress during wartime. Advocates of such treatments point to various methods that target pain, recent PTSD, and sleep disturbances collectively, including narrative exposure therapy, eye-movement desensitization and reprocessing, self-managing skills development, and low-dose NSAIDs (Lies, Jones & Ho, 2019). By taking a comprehensive approach to addressing the long-term impact of war on sleep quality, we can help to alleviate its enduring legacy and support individuals in their pursuit of health and wellbeing.

Despite the many strengths of our study, several limitations warrant mention. The VHAS is limited in its sampling and attrition rates, and generalizing our results may contribute to biased conclusions about the current health status of our participants. One example of this limitation is that our study could only include surviving older Vietnamese adults, preventing us from studying the impact of war exposure among those killed during the war or

who have died since the war ended. This selective mortality within this war era cohort may dampen the associations between war exposures and later-life health. Furthermore, our main dependent variable relied on one question assessed in participants by self-report questionnaire. Although this variable was adapted from validated self-report measures, it was not verified by biometric calculations or actigraphy and the question itself may be interpreted differently based on the participants' understanding of insomnia. To remedy this limitation, further studies could include a broader range of questions addressing sleep quality and insomnia, along with a detailed clinical assessment of sleep quality to provide a more comprehensive understanding.

This study is only the beginning of a larger, cross-sectional examination of sleep quality among Vietnamese older adults. The VHAS recently finished collecting its second wave of data in 2022, and its self-report questionnaires were revised to include additional recent sleep measures. Some examples of questions asked of the participants in Wave 2 include:

i.   "Have you experienced insomnia in the last month?"

ii.   "In the past month, how many hours and minutes did you approximately sleep on a weeknight?"

iii.   "In the past month, did you sleep badly?"

The VHAS longitudinal approach, which repeats assessments of physical and mental health status at two time points (2018 and 2022), permits analyses of changes in health linked to

disordered sleep. Further studies should compare prevalence and predictors of insomnia in other post-conflict populations to obtain a more complete understanding of the legacies of war on health and aging.

In conclusion, analyses of Vietnam's cohort of older adult war survivors yields new evidence on the myriad ways that war may increase later-life insomnia risk, and more broadly, the global burden of disease. Vietnam is not alone among LMICs undergoing struggles of economic development while also providing healthcare services to a generation of older adults whose lives were deeply affected by war. The current study takes an important step toward addressing the linkages between specific, individual-level war exposures and later-life insomnia, allowing clearer identification of groups who may benefit from treatment.

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REFERENCES

Appy, C. (2003). Patriots: The Vietnam War Remembered from All Sides. Penguin Group.

Armenta, R. F., et al. (2018). Factors associated with persistent posttraumatic stress disorder among U.S. military service members and veterans. BMC Psychiatry, 18(1), 48. https://doi.org/10.1186/s12888-018-1590-5

Bombois, S., et al. (2010). Sleep disorders in aging and dementia. The Journal of Nutrition, Health & Aging, 14(3), 212–217. https://doi.org/10.1007/s12603-010-0052-7

Endeshaw, Y. (2012). Aging, Subjective Sleep Quality, and Health Status: The Global Picture.

Sleep, 35(8), 1035–1036. https://doi.org/10.5665/sleep.1984

Ferraro, K. F., Shippee, T. P., & Schafer, M. H. (2009). Cumulative inequality theory for research on aging and the life course.Gehrman, P., et al. (20160. PTSD and Sleep. PTSD Research Quarterly, 27(4), ISSN: 1050-1835. https://www.ptsd.va.gov/publications/rq_docs/V27N4.pdf .

Gehrman, P., et al. (20160. PTSD and Sleep. PTSD Research Quarterly, 27(4), ISSN: 1050- 1835. https://www.ptsd.va.gov/publications/rq_docs/V27N4.pdf .

Gildner, T. E., et al. Associations between Sleep Duration, Sleep Quality, and Cognitive Test Performance among Older Adults from Six Middle Income Countries: Results from the Study on Global Ageing and Adult Health (SAGE). Journal of Clinical Sleep Medicine, 10(06), 613–621. https://doi.org/10.5664/jcsm.3782.

Grandner, M. A. (2017). Sleep, Health, and Society. Sleep Medicine Clinics, 12(1), 1–22. https://doi.org/10.1016/j.jsmc.2016.10.012.

Guillemot, F. (2009). Death and Suffering at First Hand: Youth Shock Brigades during the Vietnam War (1950–1975). Journal of Vietnamese Studies, 4(3), 17–60. https://doi.org/10.1525/vs.2009.4.3.17.

Hirschman, C., Preston, S., & Loi, V. M. (1995). Vietnamese casualties during the American war: A new estimate. Population and Development Review, 21(4), 783–813.

Korinek, K., et al. (2019). Design and measurement in a study of war exposure, health, and aging: protocol for the Vietnam health and aging study. BMC Public Health 19, 1351.https://doi.org/10.1186/s12889-019-7680-6

Korinek, K., et al. (2020). Is war hard on the heart? Gender, wartime stress and late life cardiovascular conditions in a population of Vietnamese older adults. Social Science & Medicine, 265. https://doi.org/10.1016/j.socscimed.2020.113380.

Kovnick, M. O., et al. (2021). The Impact of Early Life War Exposure on Mental Health among Older Adults in Northern and Central Vietnam. Journal of health and social behavior, 62(4), 526–544. https://doi.org/10.1177/00221465211039239.

Kripke, D. F., Simons, R. N., Garfinkel, L., & Hammond, E. C. (1979). Short and long sleep

and sleeping pills. Is increased mortality associated? Archives of General Psychiatry, 36(1), 103–116. https://doi.org/10.1001/archpsyc.1979.01780010109014

Kwon, H. (2012). Rethinking traumas of war. South East Asia Research, 20(2), 227–237. http://www.jstor.org/stable/23752539

Laderman, S., & Martini, E. A. (Eds.). (2013). Four Decades On: Vietnam, the United States, and the Legacies of the Second

Indochina War. Duke University Press. https://doi.org/10.2307/j.ctv11qdz12

Leeming, M. (2015). Review of Agent Orange: History, Science, and the Politics of Uncertainty, by E. Martini. Environment and History, 21(1), 165–167. http://www.jstor.org/stable/43299723

Lies, J., Jones, L., & Ho, R. (2019). The management of post-traumatic stress disorder and associated pain and sleep disturbance in refugees. BJPsych Advances, 25(3), 196–206. https://doi.org/10.1192/bja.2019.7

Medic, G., Wille, M., & Hemels, M. E. (2017). Short- and long-term health consequences of sleep disruption. Nature and Science of Sleep, 9, 151–161. https://doi.org/10.2147/NSS.S134864

Miguel, E., & Roland, G. (2011). The long-run impact of bombing Vietnam. Journal of Development Economics, 96(1), 1–15. https://doi.org/10.1016/j.jdeveco.2010.07.004.

Nguyen, H. T. (2017, January 17). Opinion | As the Earth Shook, They Stood Firm. The New York Times. https://www.nytimes.com/2017/01/17/opinion/as-the-earth-shook-they-stood- firm.html

Shepard, J. W., et al. (2005). History of the Development of Sleep Medicine in the United States. Journal of Clinical Sleep Medicine: JCSM: Official Publication of the American Academy of Sleep Medicine, 1(1), 61–82.

Simonelli, G., et al. (2018). Sleep health epidemiology in low and middle-income countries: A systematic review and meta-analysis of the prevalence of poor sleep quality and sleep duration. Sleep Health, 4(3), 239–250. https://doi.org/10.1016/j.sleh.2018.03.001

Stranges, S., et al. (2012). Sleep Problems: An Emerging Global Epidemic? Findings From the In Depth WHO-SAGE

Study Among More Than 40,000 Older Adults From 8 Countries Across Africa and Asia. Sleep, 35(8), 1173-1181. https://doi.org/10.5665/sleep.2012.

Watson, N., et al. (2015). Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society on the Recommended Amount of Sleep for a Healthy Adult: Methodology and Discussion. Sleep, 38(8), 1161-1183. https://doi.org/10.5665/sleep.4886.

Zimmer, Z., et al. (2021a). War across the life course: examining the impact of exposure to conflict on a comprehensive inventory of health measures in an aging Vietnamese population, International Journal of Epidemiology, 50,(3), 866–879. https://doi.org/10.1093/ije/dyaa247.

Zimmer, Z., et al. (2021b). Early-Life War Exposure and Later-Life Frailty Among Older Adults in Vietnam: Does War Hasten Aging?, The Journals of Gerontology: Series B, gbab190, https://doi.org/10.1093/geronb/gbab190.

TABLES & FIGURES

Table 1. Univariate and Bivariate Analyses of Insomnia Severity, Vietnamese Adults Age 59 and Older

	Total Sample % / mean	*n*	Male Subsample % / mean	*n*	Female Subsample % / mean	*n*	Chi-square, M/F test of difference
Dependent Variable: Insomnia Severity							
No insomnia in past month	30.25	*704*	35.69	*409*	24.98	*295*	0.000
Moderate insomnia in past month	47.23	*1099*	43.37	*497*	50.97	*602*	
Severe insomnia in past month	22.52	*524*	20.94	*240*	24.05	*284*	
Independent Variables							
Military Participation (3-cat)							
No Military Service	32.96	*767*	12.22	*140*	53.09	*627*	0.000
Informal Military Service	25.91	*603*	12.30	*141*	39.12	*462*	
Service in Youth Shock Programs (YSP)	8.94	*208*	5.85	*67*	11.94	*141*	
Service in Militias	15.60	*363*	5.41	*62*	25.49	*301*	
Service in both YSP & Militias	1.38	*32*	1.05	*12*	1.69	*20*	
Formal Military Service	41.13	*957*	75.48	*865*	7.79	*92*	
Wartime Exposures							
Violence Exposures (Std Index Centered on Zero)	0.013	*2327*	0.168	*1146*	-0.137	*1181*	0.000
Wounded in Warzone	21.15	*492*	35.37	*405*	7.37	*87*	
Saw dead/injured Vietnamese soldiers	52.60	*1222*	71.74	*820*	34.07	*402*	
Saw dead/injured American soldiers	25.09	*583*	40.21	*460*	10.42	*123*	
Saw dead/injured civilians	51.51	*1197*	61.80	*707*	41.53	*490*	
Know persons who died or were seriously injured	38.05	*884*	49.78	*570*	26.66	*314*	
Malevolent Conditions Exposures (Std Index Centered on Zero)	0.008	*2327*	0.025	*1146*	-0.01	*1181*	0.413
Moved due to bombing during the war	29.17	*677*	30.04	*343*	28.33	*334*	
Moved due to evacuations during the war	27.64	*643*	28.03	*321*	27.27	*322*	
Experienced illness, weakness, or discomfort due to shortage of clean water	17.19	*400*	22.08	*253*	12.45	*147*	
Experienced illness, weakness, or discomfort due to shortage of food	25.87	*602*	27.66	*317*	24.13	*285*	
Experienced inability to sleep due to noise, inhospitable conditions	45.5	*1055*	47.42	*543*	43.43	*512*	
Experienced fear of being injured or killed	34.97	*813*	29.06	*333*	40.71	*480*	
Exposed to toxic chemicals	13.81	*321*	21.92	*251*	5.94	*70*	
Recent PTSD Symptoms							
No PTSD	43.58	*1014*	39.09	*448*	47.93	*566*	0.000
A few PTSD symptoms	42.49	*984*	44.94	*515*	39.71	*469*	
Subclinical PTSD	10.92	*254*	12.04	*138*	9.82	*116*	
PTSD (provisional)	3.22	*75*	3.93	*45*	2.54	*30*	
Recent Life Stressors							
Family Stress Index	0.242	*2327*	0.226	*1146*	0.257	*1181*	0.074

Stress caused by marital disruption	3.95	*92*	2.01	*23*	5.84	*69*	
Stress caused by the death of a child	2.11	*49*	2.27	*26*	1.95	*23*	
Stress caused by a spouse's illness	27.37	*637*	28.97	*332*	25.83	*305*	
Personal Event Stress Index	0.159	*2327*	0.127	*1146*	0.191	*1181*	0.000
Stress caused by physical or psychological accident	14.400	*335*	11.78	*135*	16.93	*200*	
Stress caused by major residential move	1.380	*32*	0.96	*11*	1.78	*21*	
Financial Stress Index	0.642	*2327*	0.535	*1146*	0.743	*1181*	0.000
Stress caused by great financial difficulty	34.68	*807*	30.45	*349*	38.78	*458*	
Pain Level							
None	31.59	*735*	35.95	*412*	27.35	*323*	0.000
Mild	12.76	*297*	11.17	*128*	14.31	*169*	
Moderate	37.77	*879*	37.00	*424*	38.53	*455*	
Severe	17.88	*416*	15.88	*182*	19.81	*234*	
Frailty Level							
Not Frail	43.32	*1005*	49.08	*559*	37.76	*446*	0.000
Very Mildly Frail	10.69	*248*	10.45	*119*	10.92	*129*	
Mildly Frail	18.97	*440*	18.61	*212*	19.31	*228*	
Moderately/Severely Frail	27.03	*627*	21.86	*249*	32.01	*378*	
Physical Activity in Past Year							
None	45.60	*1061*	38.74	*444*	52.24	*617*	0.000
Infrequent physical activity	12.29	*286*	12.13	*139*	12.45	*147*	
~Daily physical activity	42.11	*980*	49.13	*563*	35.31	*417*	
Control Variables							
Age							
59-64	32.66	*760*	32.72	*375*	32.60	*385*	0.186
65-69	24.50	*570*	26.44	*303*	22.61	*267*	
70-74	16.97	*395*	16.93	*194*	17.02	*201*	
75-79	11.47	*267*	10.82	*124*	12.11	*143*	
80-84	7.95	*185*	7.50	*86*	8.38	*99*	
85+	6.45	*150*	5.58	*64*	7.28	*86*	
Sex							
Male	49.25	*1146*	--	--	--	--	
Female	50.75	*1181*	--	--	--	--	
District							
Bavi	33.91	*789*	33.77	*387*	34.04	*402*	0.996
Yen Khanh	33.26	*774*	33.16	*380*	33.36	*394*	
Dong Hoi	16.29	*379*	16.40	*188*	16.17	*191*	
Bo Trach	16.54	*385*	16.67	*191*	16.43	*194*	
Marital Status							
Not currently married	27.29	*635*	7.24	*83*	46.74	*552*	0.000
Currently married	72.71	*1692*	92.76	*1063*	53.26	*629*	
Educational Attainment							
Did not Complete HS	85.13	*1981*	79.06	*906*	91.02	*1075*	0.000
Completed HS	14.87	*346*	20.94	*240*	8.98	*106*	
Current Working Status							

No	53.29	*1240*	51.05	*585*	55.46	*655*	0.033
Yes	46.71	*1087*	48.95	*561*	44.54	*526*	

Table 2a. Generalized Ordered Logit Model Results: Moderate/Severe Insomnia (versus None)

	Model 1		Model 2		Model 3	
	Rel Odds Ratio	se	Rel Odds Ratio	se	Rel Odds Ratio	se
War Stress Exposures						
Military Service Status - Nonveteran (Omitted)	1.000	(.)	1.000	(.)	1.000	(.)
Military Service Status -Formal Military	1.169*	(0.068)	1.052	(0.067)	1.021	(0.058)
Military Service Status -Informal Military	1.444*	(0.206)	1.241	(0.154)	1.269	(0.176)
Index of Wartime Violence/Death exposures	1.208*	(0.081)	1.099	(0.068)	1.089	(0.071)
Index of Wartime Malevolent Conditions	1.155*	(0.052)	1.098	(0.056)	1.103+	(0.054)
PTSD Symptom Severity						
Recent PTSD: None, no symptoms (Omitted)			1.000	(.)	1.000	(.)
Recent PTSD: A few symptoms			1.663***	(0.108)	1.470***	(0.101)
Recent PTSD: PTSD, Subclinical			2.271**	(0.413)	1.607*	(0.255)
Recent PTSD: PTSD, Provisional			3.348**	(1.073)	2.213*	(0.732)
Recent Life Event Stressors						
Severity of Recent Family-related Stressors					1.382***	(0.089)
Severity of Recent Event Stressors					0.968	(0.091)
Severity of Recent Financial Stressors					1.192**	(0.052)
Recent Health Conditions						
Physical Exercise in Past Year: None (Omitted)					1.000	(.)
Physical Exercise in Past Year: Monthly to Weekly					0.902	(0.078)
Physical Exercise in Past Year: Daily or Almost Daily					0.846	(0.082)
Does not Report Physical Pain (Ref)					1.000	(.)
Reports Slight Pain					1.244	(0.156)
Reports Moderate Pain					1.685**	(0.202)
Reports Severe Pain					2.273***	(0.335)
Control Variables						
Current age: 59-64 (omitted)	1.000	(.)	1.000	(.)	1.000	(.)
Current age: 65-69	1.176+	(0.101)	1.136	(0.096)	1.169+	(0.093)
Current age: 70-74	0.998	(0.110)	0.986	(0.099)	1.024	(0.092)
Current age: 75-79	1.034	(0.133)	1.029	(0.127)	1.048	(0.139)
Current age: 80-84	0.802	(0.168)	0.848	(0.172)	0.926	(0.190)
Current age: 85+	0.787	(0.161)	0.815	(0.165)	0.824	(0.164)
Male (Omitted)	1.000	(.)	1.000	(.)	1.000	(.)

Female	2.147**	(0.356)	2.070**	(0.311)	1.931**	(0.320)
Bavi district (omitted)	1.000	(.)	1.000	(.)	1.000	(.)
Yen Khanh	1.359**	(0.105)	1.303**	(0.100)	1.094	(0.100)
Dong Hoi	1.033	(0.407)	1.017	(0.374)	0.920	(0.289)
Bo Trach	1.569**	(0.143)	1.561**	(0.146)	1.294*	(0.134)
Marital Status - Not Currently Married (Omitted)	1.000	(.)	1.000	(.)	1.000	(.)
Marital Status - Currently Married	1.001	(0.153)	1.012	(0.158)	1.020	(0.160)
Not presently working for pay/profit (omitted)	1.000	(.)	1.000	(.)	1.000	(.)
Currently working for pay/profit	0.757**	(0.049)	0.773**	(0.047)	0.842*	(0.053)
Educational Attainment: Secondary or Higher (Ref: LT Secondary)	0.724+	(0.121)	0.733+	(0.122)	0.860	(0.148)
N	2327		2327		2327	

Table 2b. Generalized Ordered Logit Model Results: Severe Insomnia (versus None/Moderate)

	Model 1		Model 2		Model 3	
	Rel Odds Ratio	se	Rel Odds Ratio	se	Rel Odds Ratio	se
War Stress Exposures						
Military Service Status - Nonveteran (Omitted)	1	(.)	1	(.)	1	(.)
Military Service Status -Formal Military	1.169*	0.068	1.052	-0.067	1.021	0.058
Military Service Status -Informal Military	1.444*	0.206	1.241	-0.154	1.269	0.176
Index of Wartime Violence/Death exposures	1.208*	0.081	1.099	-0.068	1.089	0.071
Index of Wartime Malevolent Conditions	1.299**	0.077	1.237**	-0.075	1.103+	0.054
PTSD Symptom Severity						
Recent PTSD: None, no symptoms (Omitted)			1	(.)	1	(.)
Recent PTSD: A few symptoms			1.663***	-0.108	1.470***	0.101
Recent PTSD: PTSD, Subclinical			2.271**	-0.413	1.607*	0.255
Recent PTSD: PTSD, Provisional			3.348**	-1.073	2.213*	0.732
Recent Life Event Stressors						
Severity of Recent Family-related Stressors					1.382**	-0.09
Severity of Recent Event Stressors					0.968	0.092
Severity of Recent Financial Stressors					1.192**	0.052
Recent Health Conditions						

Physical Exercise in Past Year: None (Omitted)					1	(.)
Physical Exercise in Past Year: Monthly to Weekly					0.902	-0.078
Physical Exercise in Past Year: Daily or Almost Daily					0.846	-0.082
Does not Report Physical Pain (Ref)					1	(.)
Reports Slight Pain					1.244	-0.156
Reports Moderate Pain					1.685**	-0.202
Reports Severe Pain					4.068***	-0.445
Control Variables						
Current age: 59-64 (omitted)	1	(.)	1	(.)	1	(.)
Current age: 65-69	1.176+	0.101	1.136	-0.096	1.169+	0.093
Current age: 70-74	0.998	-0.11	0.986	-0.099	1.024	0.092
Current age: 75-79	1.034	0.133	1.029	-0.127	1.048	0.139
Current age: 80-84	0.802	-0.168	0.848	-0.172	0.926	-0.19
Current age: 85+	0.787	0.161	0.815	-0.165	0.824	0.164
Male (Omitted)	1	(.)	1	(.)	1	(.)
Female	1.515*	0.271	1.451+	-0.245	1.31	0.238
Bavi district (omitted)	1	(.)	1	(.)	1	(.)
Yen Khanh	1.359**	0.105	1.303**	-0.1	1.094	-0.1
Dong Hoi	1.033	0.407	1.017	-0.374	0.92	0.289
Bo Trach	1.569**	0.143	1.561**	-0.146	1.294*	0.134
Marital Status - Not Currently Married (Omitted)	1	(.)	1	(.)	1	(.)
Marital Status - Currently Married	1.001	0.153	1.012	-0.158	1.02	-0.16
Not presently working for pay/profit (omitted)	1	(.)	1	(.)	1	(.)
Currently working for pay/profit	0.757**	0.049	0.773**	-0.047	0.842*	0.053
Educational Attainment: Secondary or Higher (Ref: LT Secondary)	0.724+	0.121	0.733+	-0.122	0.86	0.148
N	2327		2327		2327	

Figure 2. Study Area Map & District Level War Intensity
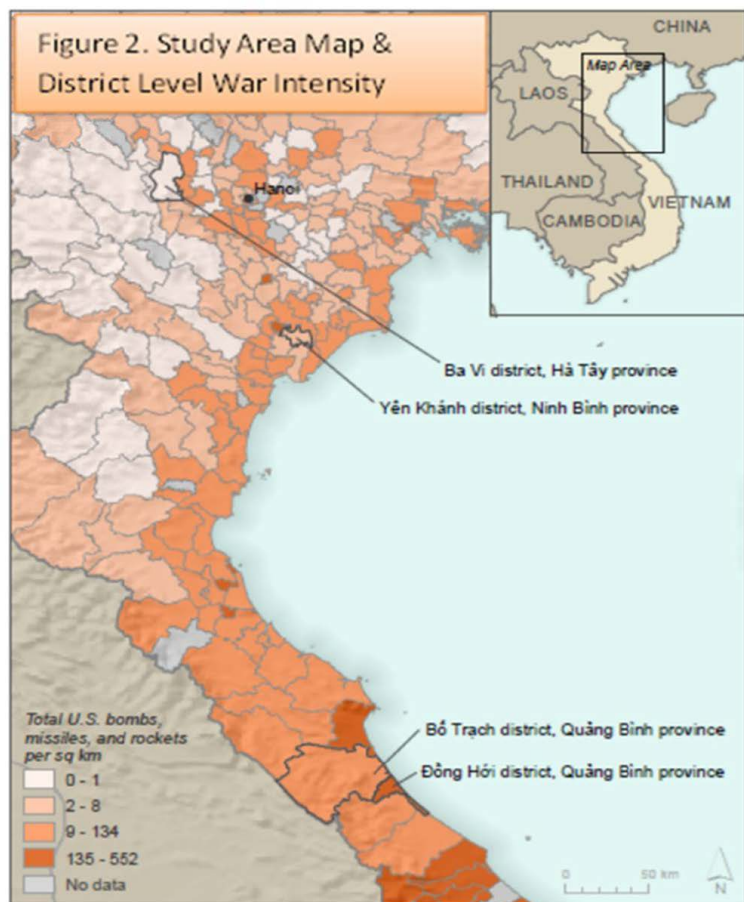
Figure 2. Insomnia Severity in the Past Month (%)

Figure 3. Military Service Background (%) by Respondent Gender

Figure 4. Indices of War-related Stressors by Insomnia Severity
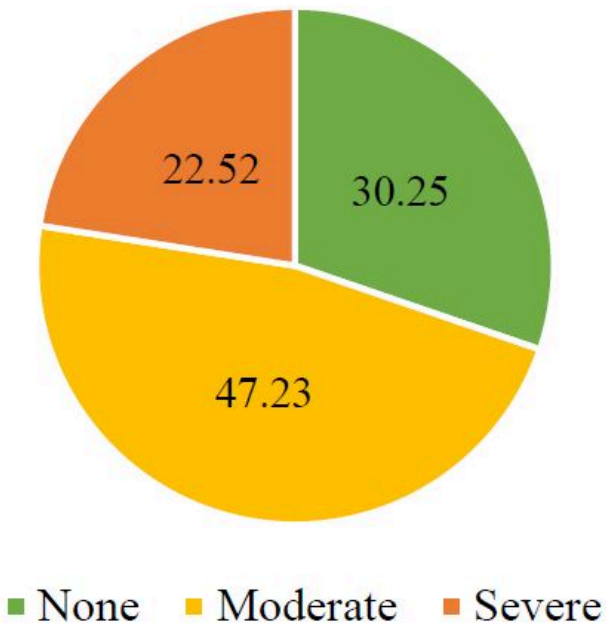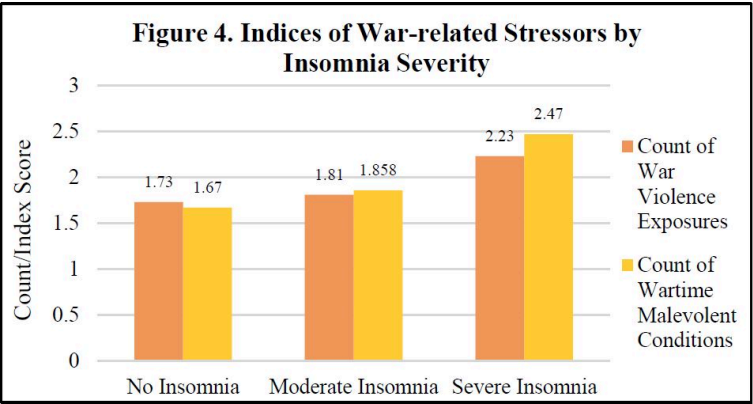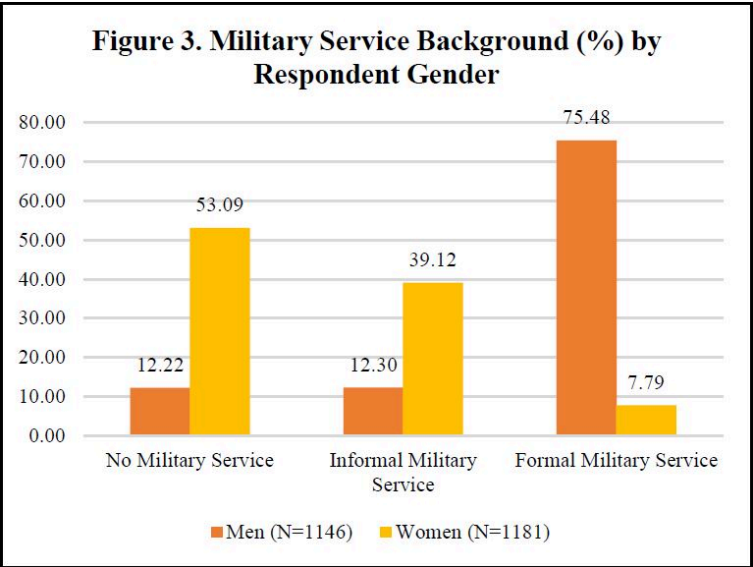
Figure 5. PTSD Symptoms by Insomnia Severity
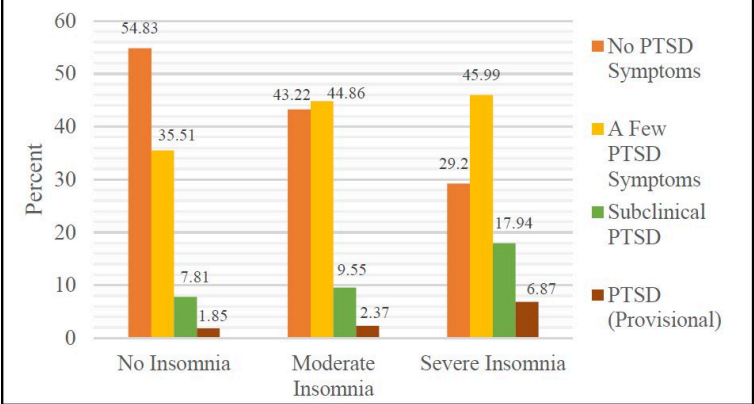
Figure 6. Indices of Recent Stressors by Insomnia Severity

Figure 7. Pain Level by Insomnia Severity

Figure 8. Frailty Level by Insomnia Severity
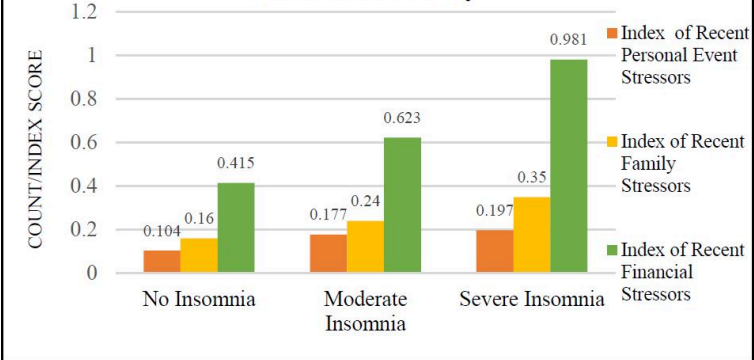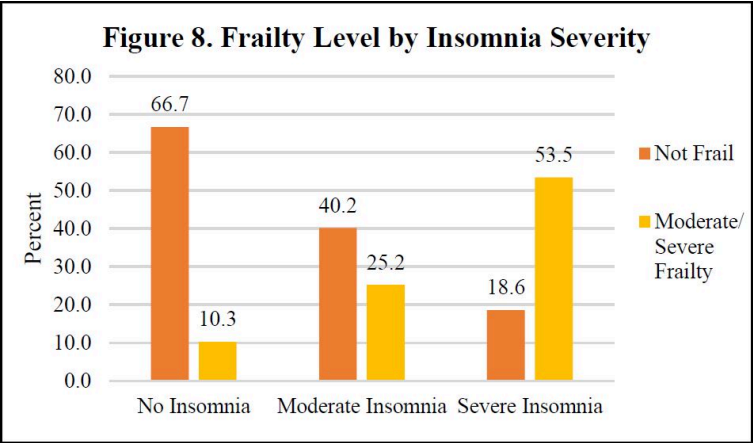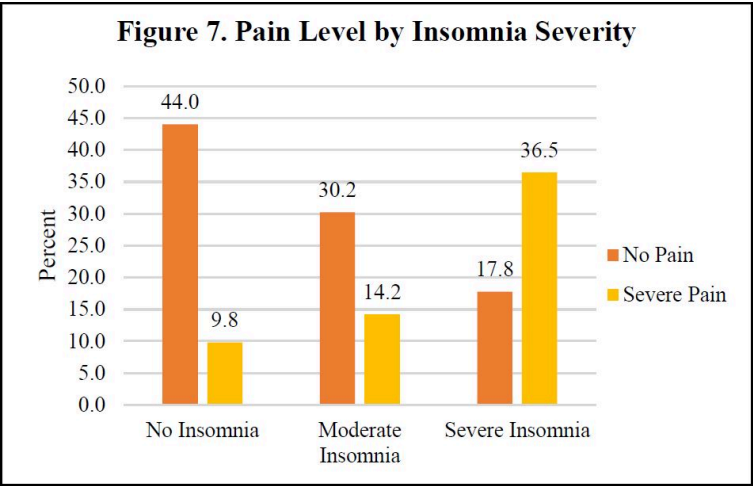
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145. **Research Reflection by Sierra Young**

Sierra Young

Faculty Mentor: Kim Korinek (Sociology, University of Utah)

My undergraduate research was the highlight of my college experience. It was a culmination of all my studies at the University of Utah and it showed me the practical application of a degree in Sociology. The skills I've learned throughout this research process will no-doubt carry over into my future career regardless of the path I take. In addition, the research I've conducted with Dr. Kim Korinek and the Vietnam Health and Aging Studies team has opened many doors of opportunity to study and intern abroad, as well as continue research post-graduation. I would recommend to any student to pursue undergraduate research, even if it doesn't end with a finished product, because the biggest value of the experience was the confidence and direction it gave me.

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SECTION XIII

Social Work

146. Mobility Consequences of Decentralizing Homeless Services and Shelters in Salt Lake County

Morrison Donovan and Sarah L. Canham (Social Work)

Faculty Mentor: Sarah L. Canham (Social Work, University of Utah)

Abstract

How homeless services, including emergency shelters, are delivered and where they are sited have significant implications for people experiencing homelessness (PEH). Being able to access necessary services is important to PEH

health, well-being, and autonomy. Using Salt Lake County, Utah, which moved from a centralized homeless shelter model to a decentralized resource center model as a case study, we investigated the impact of this transition on the mobility of PEH. We conducted in-depth, semi-structured interviews with 19 clients of three resource centers who had also previously stayed at the former shelter. Thematic analysis resulted in four broad categories, each with distinct sub-categories: 1) Pre-decentralization transportation and mobility, 2) Post-decentralization transportation and mobility, 3) Impact on quality of life, and 4) Recommendations. Participants' recommendations for improving mobility conditions included the elimination of financial barriers associated with public transportation use and expanding access to transportation networks from the resource centers.

Introduction

Utah has witnessed a concerning rise in rates of homelessness since 2016 (National Alliance to End Homelessness, 2021), which the *State of Utah Annual Report on Homelessness* attributes to the lack of available affordable housing, wage stagnation, limited employment options, and decreasing household incomes, all of which have been exacerbated by the COVID-19 pandemic (Department of Workforce Services, 2021). Homelessness is an enduring policy challenge with community-wide implications for how to address the needs of people experiencing homelessness (PEH), including the provision and maintenance of homeless shelters and services.

Decisions on where to site homeless shelters and resources face significant pressures from local interests, which has contributed to the historical and enduring concentration of homeless services in economically marginalized areas

(Brinegar, 2003). Jocoy and Del Casino (2010) identify how "spatial mismatch" occurs when PEH are isolated from community services and economic centers and the negative influence of centralized models on mobility, travel behaviors, and needs of PEH. Centralized models isolate PEH from social and economic centers and affect their transportation demands and mobility patterns (Jocoy & Del Casino, 2010).

The decentralization of homeless services has been proposed to counteract the concentration of current services and the systematic spatial exclusion of PEH from economic and social opportunities (Brinegar, 2003). In 2016, Salt Lake County, Utah began the process of transitioning from a centralized homeless shelter located downtown, known as The Road Home's Salt Lake Community Shelter and Resource Center (TRHSLC), to a decentralized model of shelter services through smaller emergency shelters called Homeless Resource Centers (HRCs). Decentralization in Salt Lake County provides a case study to investigate the impact of this model on transportation access and mobility patterns of PEH.

Research Questions:

1) How does decentralization influence transportation and mobility behaviors for PEH?

2) How have transportation and mobility changes affected access to basic services for PEH?

3) How have transportation and mobility changes resulting from decentralization affected quality-of-life among PEH?

Methods

We conducted in-depth, semi-structured qualitative interviews with PEH to address our research questions. We then used inductive qualitative analytic methods to identify patterns in the data and nuanced understandings of a relatively unexplored phenomenon (Braun & Clarke, 2006)—how

decentralization affected PEH mobility, transportation needs, and access to services. Ethics approval was obtained from the University of Utah Institutional Review Board and participant names were removed to protect identities.

Participants were recruited from the HRCs, with help from service providers in identifying clients who had also received services from TRHSLC. In total, 19 clients consented to an interview across the three HRCs. Seven interviews were conducted at the Men's Resource Center, in which all the participants identified as male, aged 22-70 years old. Six interviews were conducted at the Gail Miller Resource Center (GMRC) with three participants identifying as female and three identifying as male, aged 33-60. Six interviews were conducted at the Geraldine King Resource Center, in which all participants identified as female, aged 37-64. Twelve participants self-identified as Caucasian, and eleven had achieved the equivalence of a high school diploma or higher education. All participants were unemployed at the time of the interviews.

In-person, semi-structured interviews were conducted with participants in April and May 2021. The length of interviews ranged from 10 to 100 minutes (mean = 41 minutes). All interviews were audio-recorded and auto-transcribed by Sonix and edited by members of the research team. Participants were given $20 gift cards to local grocery or convenience stores for their participation. Inductive qualitative analytic methods were used to identify common themes in the data, using NVivo data management software. Patterns identified by the researchers were discussed during team meetings, as well as with the project advisory committee. After an iterative process of labeling and rearranging the data, a final set of themes was agreed upon by the project team.

Results

Findings were organized into three broad categories, each with distinct sub-categories (Table 1): 1) Pre-decentralization transportation and mobility; 2) Post-decentralization transportation and mobility; 3) Impact on quality of life; and 4) Recommendations to improve transportation access for PEH. Below, we present each category and sub-category with representative quotes from participants, with reference to the specific HRC where the participant was staying.

Table 1: Thematic categories, sub-categories, and definitions

Pre-decentralization transportation and mobility	
Pre-decentralization convenience of transportation and proximity to services and entertainment	Pre-decentralization, transportation services were characterized as convenient to PEH staying at the TRHSLC for their proximity to the central downtown location, relatively reliable schedule, and reduced cost barriers.
Pre-decentralization transportation challenges	Pre-decentralization challenges included the costs of transportation outside the Free Fare Zone, the limited services available within the Free Fare Zone, the challenge of walking long distances, and the time investment of using public transit.
Post-decentralization transportation and mobility	
Proximity of the HRCs to public transit	Post-decentralization, participants described the proximity of the HRCs to TRAX stations and bus stops
Availability of Advantage Shuttle	Post-decentralization, participants described the availability of an agency shuttle to use as an alternative to public transit.
Availability of daily or monthly transit passes	Post-decentralization, participants described the ability for the HRCs' case managers to provide transit passes or tokens to clients at no cost.
Challenge of the HRCs being further away from downtown and increased time commitment to use transit	Post-decentralization, participants described the HRCs' distance from the central downtown area of Salt Lake City and the subsequent increase in time invested in using public transit as a transportation challenge.

Challenge of accessing public transit for PEH with mobility limitations	Post-decentralization, participants described the difficulty for PEH with mobility limitations to walk to transit stops.
Cost barriers of using public transit outside the Free Fare Zone	Post-decentralization, participants described the cost barriers of using transit given that the HRCs are sited outside the Free Fare Zone

Impact on Quality of Life

Improved Facilities and Services through the HRCs Led to Improved Health and Quality of Life	Participants described how the decentralization process and transition in shelter systems positively impacted their quality of life. Improvements in both the physical facilities of the HRCs and the case management and resources offered to clients, and improvements in general health outcomes led to reports of improved quality of life among PEH.
Mobility Barriers Associated with HRCs Negatively Impact Quality of Life	Despite the improvements in facilities, services, and health outcomes, barriers to mobility from the HRCs were reported to negatively affect quality of life post-decentralization.
Transportation Access Represents an Escape	The shelter environment can represent a constraint to well-being by constraining individual freedoms, and transportation access and freedom in mobility serves as a remedy.

Recommendations

Eliminate cost barriers to transportation for PEH	To eliminate cost barriers to transportation, participants made recommendations to provide free transit, to provide HRC clients unlimited transit linked to their services card, to expand the capacity of HRCs to offer transit passes, and to base the cost of transit on a person's income.

Increase access to transportation from HRCs	Recommendations for increasing access to transportation from the HRCs included expanding UTA bus service, the Advantage Shuttle service, and the Free Fare Zone.

In addition to the themes identified in the table, an additional theme, identified is the idea that transportation access and freedom in mobility represent an escape from the shelter environment and a way for PEH to retain a sense of normalcy in their lives. Quality of life was significantly negatively impacted by reduced mobility for many participants. One participant explained the impact of their reduced mobility:

Some of us used to liken [TRHSLC] to a concentration camp, because you couldn't go anywhere without showing a card... what little bit of freedom you did have, it seemed like you lost it... I rode TRAX a lot because I could leave. I could go from the Road Home right up to the library, I could still have a little bit of normalcy... I don't [go to the library anymore], I can't get to it... Being stuck in a shelter, not good, but I think this one's [the GMRC is] actually worse than the Road Home was because like I said, I could get around more, I could actually get out of it.

Discussion and Conclusions

The findings of this study contribute to the existing body of literature on the relationships between homeless service delivery systems, PEH's access to economic and social opportunities, and the importance of access to affordable transportation networks among PEH. Investigating the impact of decentralizing homeless shelters and services in Salt Lake County, Utah on mobility and transportation use among PEH revealed a few consistent trends: transportation access was described as generally more convenient from the centralized system, with fewer associated cost burdens, and challenges

to mobility post-decentralization included increased time and financial investments to access services. The respondents' quality of life was positively impacted by facility improvements that were conducive to better health outcomes, and negatively impacted by the post-decentralization constraints to mobility. Eliminating cost barriers to public transportation networks and expanding UTA access from the HRC sites were the primary recommendations offered to improve PEH's mobility post-decentralization.

The location of homeless resources and shelters also has important implications for well-being among PEH because of the impact that shelter locations have on mobility and the need for transportation. While mobility and access to public transportation has long been acknowledged as a factor that can contribute to social isolation and exclusion, mobility has been more broadly connected to one's well-being through its effect on people's ability to fulfill psychological needs (Stanley et al., 2011). Stanley et al. (2011) suggest that travel exerts an indirect influence on well-being through its direct impact on social exclusion. This is supported by the findings reported by participants who encountered challenges and barriers to transportation, destinations, and services. The negative impact of barriers to mobility were cited as the biggest change in many people's lives after the transition. Because mobility is a cornerstone of self-determination and many of the psychological dimensions of well-being, a reduction in mobility represents a big life change for PEH, who already encounter significant disruption and instability. Transportation access and mobility are central to well-being as they represent not only an escape from the shelter environments, but a way to retain a sense of normalcy, autonomy, and routine.

To combat the economic and social marginalization of

homeless populations that can be wrought by centralized and decentralized models of homeless shelter service delivery systems alike, transportation access must be a key policy priority and component of siting decisions. Participants offered several recommendations that strengthen the evidence that transportation access and freedom are central to well-being. Eliminating cost barriers was identified as a key priority by participants, which could include expanding the Free Fare Zone, allowing HRC clients free transit through their services card, expanding the availability of tokens and transit passes offered by HRC case managers, or adjusting transit costs to account for a person's income. These recommendations implicate the need for coordination between UTA and the HRCs to rectify the mobility challenges posed by the locations of the HRCs while prioritizing free public transit access to clients, who are now located beyond the Free Fare Zone. Other recommendations participants made included expanding transportation access from the HRCs, including expansion of UTA services, specifically by increasing the frequency of transit stops and expanding the availability of UTA buses to transport PEH to TRAX or the Free Fare Zone. Expanded frequency and flexibility of shuttle services were also described as a remedy to the transportation challenges associated with the HRCs. Transporting PEH to the other HRCs or to TRAX and the Free Fare Zone would create a more affordable and convenient transportation network.

References

Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology, 3,* 77-101. https://doi.org/10.1191/1478088706qp063oa

Brinegar, S. J. (2003) The social construction of homeless

shelters in the Phoenix area. *Urban Geography, 24*(1), 61-74, https://doi.org/10.2747/0272-3638.24.1.61

Cleveland, A. (2016). *Summary of neighborhood engagement workshops.* Available: http://www.slcdocs.com/ced/HRC/NEW_writeup_AC.pdf

Hoch, C. (1991). The spatial organization of the urban homeless: A case study of Chicago. *Urban Geography, 2*, 137-154.

Jocoy, C.L., & Del Casino, V.J. (2010). Homelessness, travel behavior, and the politics of transportation mobilities in Long Beach, California. *Environment and Planning A, 42*(8), 1943-1963. https://doi.org/10.1068/a42341

Lee, B. A., & Price-Spratlen, T. (2004). The geography of homelessness in American communities: Concentration or dispersion? *City and Community, 3*(1), 3–27. https://doi.org/10.1111/j.1535-6841.2004.00064.x

National Alliance to End Homelessness. *State of Homelessness: 2021 Edition: Utah; 2021.* Available: https://endhomelessness.org/homelessness-in-america/homelessness-statistics/state-of-homelessness-dashboards/?State=Utah.

Sanchez, T.W., & Schweitzer, L. (2008). Assessing federal employment accessibility policy: An analysis of the JARC Program. Available: http://www.brookings.edu/~/media/Files/rc/reports/2008/0221_transportation_sanchez/0221_transportation_sanchez.pdf

Stanley, J. K., & Hensher, D. A. (2011). Mobility, social exclusion and well-being: Exploring the links. *Transportation Research Part A: Policy and Practice, 45*(8), 789-801. https://doi.org/10.1016/j.tra.2011.06.007

Utah Homeless Management Information System. (2016).

Point in Time (PIT) Report. Available: https://utahhmis.org/reports/trends-in-homelessness/

United States Interagency Council on Homelessness. (2020). *Utah Homelessness Statistics.* Available: https://www.usich.gov/homelessness-statistics/ut

Workforce Services: Homeless Services. (2021). *State of Utah Annual Report on Homelessness 2021.* Available: https://jobs.utah.gov/homelessness/homelessnessreport.pdf

Wu, B. M., & Hine, J. P. (2003). A PTAL approach to measuring changes in bus service accessibility. *Transport Policy, 10*(4), 307-320. https://doi.org/10.1016/S0967-070X(03)00053-2

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147. **Alternative Justice Policies for Nonviolent Drug Offenses: A Policy Analysis**

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Issue Statement

The US incarcerates the most people globally, resulting in approximately 2,000,000 incarcerated people annually (Sawyer & Wagner, 2022; Vera Institute on Justice, n.d.). Of the 2,000,000 incarcerated people, 373,500 (19%) are incarcerated for drug offenses (Sawyer & Wagner, 2022). A drug offense is a nonviolent offense that includes substance possession, driving

under the influence, minor trafficking, sales, and manufacturing (Sawyer & Wagner, 2022; Mauer & King, 2007). In 2020, there were 1,155,610 drug arrests, the vast majority of which (87.7%) were for drug possession or use rather than sale or manufacturing (Sawyer & Wagner, 2022). Thus, many drug offenders become incarcerated for possessing and using substances. The cost of incarcerating drug offenders is 56.6 billion dollars annually, and the effectiveness of incarceration is minimal (The Sentencing Project, 2021). The cost to incarcerate drug offenders is three times greater than the benefit to public safety, with every 1$ spent on incarceration yielding a $0.35 safety benefit (Henrichson, 2013). Additionally, incarcerating drug offenders is an ineffective intervention because drug offenders have a 57.7% recidivism rate for drug offenses alone over ten years post-release, resulting in nearly three of five drug offenders becoming reincarcerated for drug offenses (Antenangeli et al., 2021).

Moreover, significant drug offender populations exist at all US correctional levels, including juvenile detention centers, jails, state prisons, federal prisons, military prisons, and immigration detention centers (Sawyer & Wagner, 2022). The correctional facilities with the highest concentration of convicted drug offenders are state prisons (146,000) and federal prisons (67,000) (Sawyer & Wagner, 2022). Drug offenders in state prisons compose 14% of the inmate population (Carson, 2021). The demographics of state prison drug offenders are female (26%), male (13.1%), White (16.6%), Black (12.2%), Hispanic (11.7%), Asian (11%), and American Indian/Alaskan Native (10.9%) (Carson, 2021). The state inmate groups with the most significant drug offender population are aged 30-39 (Mississippi Department of Corrections, 2021; Edwards & Le Blanc, 2022). In contrast, drug offenders in federal prisons

compose 46.7% of the inmate population (Carson, 2021). The demographics of federal prison drug offenders are female (61.6%), male (45.6%), Hispanic (62%), Asian (45.7%), Black (42.1%), White (39.2%), and American Indian/Alaska Native (16.3%) (Carson, 2021). The federal inmate groups with the most significant drug offender populations are aged 30-39 (Taxy et al., 2015).

Root Cause of the Issue

The 1980s War on Drugs was the US government's initiative to "crackdown" on drug crimes and criminalize drug use that had reached an apex in the 1970s counterculture and Vietnam War, which continued to grow with the rise of cocaine and crack cocaine during the 1980s (Mauer & King, 2007). The War on Drugs set the precedence for the criminal justice system (CJS) to punish and incarcerate low-level drug offenders, and the effects of the war are still apparent in the US prison system (Mauer & King, 2022; Sawyer & Wagner, 2022). The War on Drugs increased police enforcement and prosecution of drug-related offenses, which disproportionately affected low-income communities and people of color (Mauer & King, 2007). Although marginalized communities were impacted the most, the US government stigmatized illicit substance use among all groups during this period (Orsini, 2017). The CJS targeted people who were addicted to substances or used substances recreationally. Once nonviolent drug offenders were arrested, exiting the CJS became increasingly challenging due to additional systemic barriers to employment, housing, and education (Chandler et

al., 2009). Thus, many drug offenders became stuck in a justice-involved cycle.

Moreover, the War on Drugs contributed to the Anti-Drug Abuse Acts of 1986 and 1988 (Mauer & King, 2007). These laws enacted mandatory minimum sentencing for drug offenses and impacted the federal sentencing guidelines for drug offenders (Mauer & King, 2007). According to Mauer and King (2007), these policies removed judges' discretion in drug cases. The policies automatically sent drug offenders to prison to serve their entire sentence if their offense met the mandatory minimum criteria (Mauer & King, 2007). Mandatory minimum sentencing would increase drug offender incarceration rates in the prison system from 79% to 94% between 1988 to 2004 (Mauer & King, 2007). By 2004, drug offenders with mandatory minimum sentences would serve three times longer incarceration sentences than in 1988 (Mauer & King, 2007). The CJS was determined to arrest more drug offenders and incarcerate them for longer. The CJS prerogative caused a 500% increase in incarceration rates over 25 years leading into the early 2000s when previous drug policies fell under public scrutiny and began to shift toward alternatives (Mauer & King, 2007; National Centers for Drug Abuse Statistics [NCDAS], n.d.).

Implications

Mass incarceration affects individuals, families, and communities (Wilderman & Wang, 2017).

Research has shown that criminalizing people for lower-level offenses is a widespread issue that impacts many groups within society (Mauer & King, 2007; Wilderman & Wang, 2017). As society continues to confine people inside US jails and prisons, we see several issues arise, such as increased government spending, overpopulated prisons, and employment obstacles. Especially for people of color, the research shows African Americans are overrepresented in prison populations by nearly "5 times the rate of white Americans" (Nellis, 2021, p. 6). Mass imprisonment causes damage to the individual's social networks, disrupts established social norms, and distorts social norms (Roberts, 2004, p. 1281).

Due to overpopulated prisons, an increased amount of government spending directly goes to our prison and jail systems. About 7.7 million Americans have been imprisoned, 12.1 million have been sentenced to a felony, and nearly 45 million have been charged with at least one misdemeanor (Craigie et al., 2020). To manage mass incarceration, our government has increased the amount of government spending. In fact, about $80 billion is spent in the US to manage 1.7 million prisoners and supervise approximately 4 million people on probation and parole (Eisen & Stroud, 2022). The federal government continues to spend billions of dollars on highly disciplinary practices concentrated on prisons that are yet to be proven

effective. In fact, within three years of a release date, "...two out of three former prisoners are rearrested, and more than 50% are incarcerated again" (Benecchi, 2021).

The overwhelming majority of prisoners are returning. Thus the government spends more on the CJS when the funds would be more effective for organizations, agencies, or communities to reduce mass incarceration. Employment for formerly incarcerated individuals is also a problematic barrier that impairs individuals' social reintegration and post-release success. Formerly incarcerated people lose about half their earning potential resulting in a $100,000 difference compared to a non-incarcerated person (Eisen & Stroud, 2022). Additionally, many lost opportunities, insufficient reentry services, and social stigma can contribute to this connection between imprisonment and employment.

Policy Solutions Decriminalization of Illicit Substances

The policy recommendation is to remove the legal penalties for illicit substance possession and use (Drug Policy Alliance, 2017). Under this policy, the CJS will not arrest, charge, or fine people for illicit substance use or possessing personal amounts (small amounts) of illicit substances (Drug Policy Alliance, 2017). Instead, the CJS will connect people caught using or possessing illicit substances to social services like SUD treatment agencies and

harm reduction organizations for substance misuse support and education (Drug Policy Alliance, 2017). The premise is that substance misuse is a health issue and requires a medical response.

Substance decriminalization would allow the US to reduce arrests, incarceration, recidivism rates, and ethnic disparities in the CJS (Goncalves et al., 2015; Sawyer & Wagner, 2022; Drug Policy Alliance, 2017). Moreover, reduce public expenditure on drug-related incarceration and justice courts (Goncalves et al., 2015). Thus, society could spend more public money on alternatives to incarceration, like prevention, SUD treatment, education, public health, and harm reduction (Drug Policy Alliance, 2017).

Pretrial Diversion

Pretrial diversion is one of our solutions to address underlying problems by involving legal professionals to reduce contact in jails and prisons. The pretrial diversion services are intended to divert offenders from the traditional criminal justice system to a supervised program with services operated by the US Probation Services. Typically, participants who complete the program are not charged, and if charged, participants will have charges dismissed. Participants who do not successfully complete the program usually return for prosecution. (US Department of Justice Manual, 2020). Furthermore, there are countless statistics revealing the success rate of pretrial diversion

programs. The Eastern District of New York's Pretrial Opportunity Program reported a 76% success rate (American College of Trail Lawyers, 2021). The "Conviction and Sentence Alternatives" program reported an 86% of successful graduates from their program (American College of Trail Lawyers, 2021). The list of successful completion of pretrial diversion programs continues, confirming that pretrial diversion works in our society.

Community Service Restitution Program

The policy recommendation is to use community service instead of incarceration for drug offenders. Community service is a restorative justice intervention where the justice court mandates drug offenders to volunteer time at nonprofit and community organizations. The justice court determines how many community service hours offenders must complete based on the drug offense's severity and impact. Community service is a safe intervention and sentencing option when accompanied by a condition of court supervision or probation (Patchin & Keveles, 2004). Community service is a solution that enables drug offenders to comply with the courts while learning valuable skills and improving their community, self-esteem, and SUD recovery (Bouffard & Muftic, 2007). According to Picard et al. (2019), justice courts can use community service to intervene in 76% of low-level drug cases, supporting the intervention's widespread utility. At the same time, research

studies have found that incorporating a community-based program as part of treatment has resulted in a 10% reduction in low-level drug offender incarceration rates (Patchin & Keveles, 2004). Furthermore, a common approach in assessing whether implementing community service will reduce the impact of drug offender incarceration is known as the SARA Model (Patchin & Keveles, 2004). The model will be beneficial in evaluating the policy recommendation.

Part II: Issue Description

The United States incarcerates two million people; of the two million people, about 20 percent of those incarcerated are charged with drug-related offenses (Sawyer & Wagner, 2022). The issue of mass incarceration for lower-level offenses impacts individuals, families, and communities. The solutions suggested to solve the issue of mass incarceration include: decriminalizing illicit substances, pretrial diversion, and community service instead of incarceration.

Alternative Solutions

Solution 1: Decriminalize Illicit Substances

The first solution is to decriminalize illicit substances. This option would discontinue fines, incarceration, probation, or other criminal penalties for persons using or possessing illicit substances.

Solution 2: Pretrial Diversion

The second solution is pretrial diversion. These programs include legal professionals and involve programs people can turn to rather than incarceration.

Solution 3: Community Service Restitution Program

Is a restorative justice intervention where the offender is sanctioned to repay the community for the harm they caused by volunteering at nonprofit organizations and may involve paying fines.

Criteria

We have chosen effectiveness, cost-efficiency, and social acceptability as criteria for our issue. Outcome effectiveness looks at how much the option will decrease or reduce the incarceration of drug offenders. For cost-efficiency, we will analyze how much money the policy will save the United States government. Lastly, social acceptability will examine how much society, communities, and the general population will accept, agree, and/or support the policy. From our criteria, we weighted effectiveness as the highest (.4), with cost-efficiency (.3) and social acceptability (.3) with the same weight. The criteria will be used to rate different solutions by analyzing data from different resources.

Policy Analysis Solution 1: Decriminalize Illicit Substances Outcome Effectiveness

According to Drug Policy Alliance (2015), Portugal has reduced drug arrest rates by 60% and drug-related incarceration rates by 20%. Other locations around the globe exhibit data supporting decriminalization policies and programs. Germany reported a 30% decrease in criminal drug offense cases and 7% in criminal drug trials two years after implementing decriminalization (Fischer, 1995). In the U.S., Seattle, WA reported a 88% decrease in

prison incarceration sentences after implementing Law Enforcement Assisted Diversion, a decriminalization policing program (Collins et al., 2019). Furthermore, a year after Oregon passed decriminalization legislation, it reduced 9,000 drug arrests and decreased drug offender incarceration rates (Sutton, 2021). By the US implementing a policy like Portugal, estimated annual drug arrests can decrease from 1.1 million to 462,244 and reduce the 373,500 incarcerated drug offender population to approximately 298,800, if not more (Drug Policy Alliance, 2015; Goncalves et al., 2015, Sawyer & Wagner, 2022). Moreover, the policy could decrease drug offender recidivism because 57.7% of drug offenders become reincarcerated due to drug offenses like substance use and possession (Antenangeli et al., 2021; Drug Policy, 2017). Since the policy can reduce the massive flow of drug offenders entering and reentering incarceration, the policy can effectively reduce drug offender incarceration rates (Drug Policy Alliance, 2015; Drug Policy Alliance, 2017; Goncalves et al., 2015; Collins et al., 2019).

Cost-Efficiency

According to Miron and Waldock (2010), the combined state expenditures for policing substance possession crimes alone is $4.28 billion annually. Moreover, the US spends approximately $56.6 billion annually to incarcerate low-level drug offenders (The Sentencing Project, 2021).

Decriminalization would significantly reduce these costs. Oregon policy analysts estimate that Oregon will save between $12 million and $48.6 million by ending drug offender arrests, convictions, and incarceration (Martinez, n.d.). Similarly, Portugal saved $40 million over ten years post-decriminalization and reduced its governmental spending on drug-related costs by 18% (Goncalves et al., 2015). Based on the limited available literature on governmental savings related to decriminalization, by implementing a nationwide policy, the US could save between $600 million and $2.4 billion (Drug Policy Alliance, 2017; Miron & Waldock, 2010; Goncalves et al., 2015).

Social Acceptability

US society supports substance decriminalization now more than ever (Drug Policy Alliance, 2017; Rouhani et al., 2022; Franklin, 2021; Viars, 2016). According to two nationally representative surveys taken in 2021 and 2022, many US citizens support the decriminalization policy (Rouhani et al., 2022; Franklin, 2021). Depending on the survey source, support for the policy ranges from 35% to 66% of the US adult population, which translates to between 116 million to 219 million people (Rouhani et al., 2022; Franklin, 2021). The demographics with the most support for the policy are 18-34 years old (42%), male (38.3%), White, non-Hispanic (38%), college graduates (36.5%), and liberal (47%) (Rouhani et al., 2022). In 2020, Oregon

decriminalized all substances (Sutton, 2021). Following the enactment of Oregon's policy, other states like Washington, Massachusetts, Vermont, Maine, New York, Rhode Island, Maryland, Kansas, the District of Columbia, and even the United States Congress have introduced bills or started campaigns to remove the legal penalties for drug possession and increase SUD treatment access (Sutton, 2021). Two components that contribute to the policy's social acceptability are the racial disparities of people of color in drug arrests and incarceration rates and the growing support for harm reduction and treatment to reduce substance misuse, addiction, and overdose rates (Drug Policy Alliance, 2017; Rouhani et al., 2022; Franklin, 2021).

Solution 2: Pretrial Diversion

Outcome Effectiveness

The 2018 natural experiments in Harris County, Texas, revealed diversion programs decrease the likelihood of future incarceration by 48%, and the overall total of future conviction rates declined by 66% (Mueller-Smith & Schnepel, 2018). With the decline of incarceration rates, employment rates rise, and earnings increase. The research shows "Quarterly employment rates improve by 53 percent and total earnings by 64 percent" (Mueller-Smith & Schnepel, 2018, p. 2). The implementation of pretrial diversion programs positively impacts recidivism and the labor market. In addition, "diversion permanently changes an individual's

lifetime trajectory" (Mueller-Smith & Schnepel, 2018, p. 2). The implementation of pretrial diversion programs has proven to reduce incarceration rates (Rempel et al., 2018).

Cost Efficiency

The United States Criminal Justice System would save $4.8 billion if only 10% of eligible offenders were sent to diversion programs rather than prison (Kubic & Pendergrass, 2017). Specifically, San Francisco expects $3 million in savings per year projected from "a cost- benefit analysis of expanding pretrial diversion" (Pretrial Justice Institute, 2017). Broward County, Florida, estimates over $125 million in savings by diverting 20% from jail and employing existing programs such as pretrial diversion programs (Piquero, 2010). Research also shows in Santa Clara County, California, a validated pretrial risk assessment tool saved $33 million by keeping 1,400 defendants out of jail within six months (Board of Supervisors of the County of Santa Clara, 2012). Overall, the United States could save $78 billion from "systematically fine-tuning pretrial detention decisions through [a] cost-benefit analysis..." (Baughman, 2017).

Social Acceptability

Society has shown interest in pretrial diversion programs, partially driven by the mass incarceration crisis, an increase in funding, an emphasis on community prosecution, and reliable

research results (Lowry & Kerodal, 2019). Pretrial diversion programs have been authorized in 48 states and the District of Columbia (NCSL, 2017). The programs are becoming more attractive as our societies, communities, and the general population understands the potential of reduced recidivism rates and savings resulting from pretrial diversion programs (Lowry & Kerodal, 2019). In addition, 43 out of the 48 states with authorized pretrial diversion programs have population-specific diversion programs, which include programs serving: substance abuse, mental illness, domestic relations, first-time violators, defendants of property offenses, victims of human trafficking, and homeless defendants (NCSL, 2017). The information suggests that societies, communities, and the general population accept the implementation of Pretrial Diversion Programs.

Solution 3: Community Service Restitution Program

Outcome Effectiveness

There is little research on how community service restitution programs decrease or reduce incarceration rates. One study concluded with no evidence of reducing crime after completing community service restitution programs (McDonald, 1988). However, in the same study, research suggests community service sentences positively affect juveniles (McDonald, 1988). In another study, research revealed that offenders who

participate in community services sentences are less likely to encounter post-program recidivism (Bouffard & Muftic, 2007). The results of this study were captured by a sample of 200 offenders sentenced to community service, compared to 222 offenders sentenced to pay a monetary fine. The study then compared the samples to examine the effectiveness of community service sentences, revealing a decrease in recidivism rates.

Cost-Efficiency

According to Cooper and West (1981), it costs $524,000 annually to fund a community service restitution program (CSRP) for 300 offenders. The CSRP costs approximately $5,500 annually per offender (Cooper & West, 1981; Marion, 2002). In contrast, the national average annual cost to incarcerate an offender in jails is approximately $47,000 (Henrichson et al., 2015). Therefore, the CSRP can save government funding approximately $41,500 annually per offender compared to incarceration. Moreover, US jails have 113,000 non-convicted low-level drug offenders, approximately 99,101 (87.7%) of which would be eligible for a CSRP because of illicit substance use or possession violations (Cooper & West, 1981; Marion, 2002; Sawyer & Wagner, 2022). For the US to serve 99,101 drug offenders, it must create 331 CSRPs costing 173.4 million dollars annually. In contrast, the annual cost to incarcerate 99,101 drug offenders is $4.6 billion. The US could save $4.48 billion by eliminating low-

level drug offender incarceration and implementing a CSRP policy (Sawyer & Wagner, 2022, Marion, 2002; Cooper & West, 1981, Henrichson et al., 2015).

Social Acceptability

According to Families Against Mandatory Minimums (n.d.), 77% of Americans believe that alternatives to incarceration like CSRPS, probation, and rehabilitative services are the most appropriate sentence for nonviolent, non-serious offenders. Many communities, institutions, and states support CSRPs for minor, nonviolent offenses and view the programs as a positive paradigm shift away from punitive justice practices like incarceration (Wood, 2013; Karp et al., 2004; Dzur, 2011). CSRPs typically include low-level drug offenses and are available to adults and youth offenders who commonly accept CSRPs instead of incarceration (Karp et al., 2004; Wood, 2013). Justice courts throughout the US have used CSRPs as intermediate sanctions since the 1980s (Wood, 2013; Dzur, 2011). Moreover, nonprofit organizations all over the US support CSRPS and are the primary providers and facilitators of offender community service hours (Wood, 2013). These organizations benefit by receiving free labor while supporting local community members in resolving their sanctions. Some states endorse CSRPs more than others. According to Dzur (2011), the Vermont Department of Corrections incorporated 77 volunteer- based community boards throughout Vermont to

facilitate CSRPS. Plus, CSRPs have significant buy-in from victims and offenders, highlighting the support for these programs from parties on both sides of the offense (Karp et al., 2004; Dzur, 2011). According to Karp et al. (2004), 92% of victims want these programs to continue, and 72% are satisfied with the CRSP results. In contrast, offender buy-in is exhibited by an 81% program completion rate and offender participation when given the option of a CRSP or incarceration (Karp et al., 2004).

	Criteria			
Alternative Options	**Effectiveness (.4)**	**Cost-efficiency (.3)**	**Social Acceptability (.3)**	**Total (1.0)**
Decriminalize Illicit Substances	7.5 (.4) = 3	7.5 (.3) = 2.25	7 (.3) = 2.1	7.35
Pretrial Diversion	7 (.4) = 2.8	7.5 (.3) = 2.25	7.5 (.3) = 2.25	7.3
Community Service Restitution Program	5 (.4) = 2	6 (.3) = 1.8	7 (.3) = 2.1	5.9

Recommendation

The US incarcerates approximately 400,000 nonviolent drug offenders and arrests 1.15 million for nonviolent drug offenses annually (Sawyer & Wagner, 2021). Arresting and incarcerating nonviolent drug offenders is costly and ineffective (Sawyer & Wagner, 2021; The Sentencing Project, 2021; Henrichson, 2013; Antenangeli et al., 2021). There are several solutions to this

issue. For the purpose of this policy analysis, analysts selected decriminalization of illicit substances, pretrial diversion, and community service restitution programs as viable solutions for nonviolent drug offender mass incarceration. Analysts applied criteria that included outcome effectiveness, cost-efficiency, and social acceptability in rating the viability of these solutions. The final analysis rating was the decriminalization of illicit substances (7.35), pre-trail diversion (7.3), and community service restitution programs (5.8). The recommendation based on the policy analysis was decriminalizing illicit substances.

Of the three options decriminalization of illicit substances was the highest rated option.

Decriminalization is outcome effective because it significantly reduces drug arrests, drug offender incarceration, and drug-related recidivism rates. The solution will eliminate more than a million people with nonviolent minor drug offenses from criminal justice system involvement. It will help jails and prisons run more efficiently and allow justice courts and probation agencies to exert more resources on violent offenders who pose a greater risk to society than nonviolent drug offenders. Decriminalization is cost-efficient by eliminating nonviolent drug offender policing, incarceration, and court costs, which expend government agencies' funding with no notable public safety benefit. The US can expect to save billions of dollars by implementing this solution. Moreover, citizen support for decriminalization is widespread, and multiple state governments support this solution.

References American College of Trail Lawyers. (2021). *The effective use of pretrial diversion in criminal cases.* https://www.actl.com/docs/default-source/default-document-library/position-   statements-and-whitepapers/effective_use_of_pretrial_diversion_in_criminal_cases.pdf?sfvrsn=afc18679_4

Antenangeli, L., & Durose, M. R. (2021, September). *Recidivism of prisoners released in 24 states in 2008: A 10-Year follow-Up period (2008-2018).* US Department of Justice. https://bjs.ojp.gov/BJS_PUB/rpr24s0810yfup0818/Web%20content/508%20compliant%20PDFs

Baughman, S. B. (2016). Costs of pretrial detention. *Boston University Law Review, 97*(1), 1-30. https://ssrn.com/abstract=2757251

Benecchi, L. (2021, August 8). *Recidivism imprisons American progress.* Harvard Political Review. https://harvardpolitics.com/recidivism-american-progress/

Bouffard, J. A., & Muftić, L. R. (2007). The effectiveness of community service sentences compared to traditional fines for low-level offenders. *The Prison Journal, 87*(2), 171–194. https://doi.org/10.1177/0032885507303741

Carson, E. A. (2021, December). *Prisoners in 2020 – Statistical tables.* US Department of Justice. https://bjs.ojp.gov/content/pub/pdf/p20st.pdf

Chandler, R. K., Fletcher, B. W., & Volkow, N. D. (2009). Treating drug abuse and addiction in the criminal justice system: Improving public health and safety. *Jama, 301*(2), 183-190. https://doi.org/10.1001/jama.2008.976

Collins, S. E., Lonczak, H. S., & Clifasefi, S. L. (2019). Seattle's law enforcement assisted diversion (lead): Program effects on criminal justice and legal system utilization and

costs. *Journal of Experimental Criminology, 15*(2), 201-211. https://doi:10.1007/s11292-019-09352-7

Craigie, T., Grawert, A., Stiglitz, J., & Kimble, C. (2022, September 16). *Conviction, imprisonment, and lost earnings: How involvement with the criminal justice system deepens inequality.* Brennan Center for Justice. https://www.brennancenter.org/our-work/research-reports/conviction-imprisonment-and-lost-earnings-how-involvement-criminal

Drug Policy Alliance. (2015, February). *Drug decriminalization in Portugal: A health-Centered approach.* We are the Drug Policy Alliance. https://drugpolicy.org/sites/default/files/DPA_Fact_Sheet_Portugal_Decriminalization_Feb2015.pdf#:~:text=The%20number%20of%20people%20arrested%20and%20sent%20to,commissions%20are%20deemed%20non-problematic%20and%20dismissed%20without%20sanction.12

Drug Policy Alliance. (2017, July). *It's time for the US to decriminalize drug use and possession.* We are the Drug Policy Alliance. https://drugpolicy.org/sites/default/files/documents/Drug_Policy_Alliance_Time_to_Decriminalize_Report_July_2017.pdf

Dzur, A. W. (2011). Restorative justice and democracy: Fostering public accountability for criminal justice. *Contemporary Justice Review, 14*(4), 367-381. https://doi.org/10.1080/10282580.2011.616367

Edwards, J. B., & Le Blanc, J. M. (2022, July). *Briefing book.* Louisiana Public Safety & Corrections. https://s32082.pcdn.co/wp-content/uploads/2022/09/0m-Full-Briefing-Book-July-2022-Website.pdf

Eisen, L., & Stroud, H. (2022, August 5). *Securing public safety without mass incarceration or*

deepening   racial   injustice. The   Hill.
https://thehill.com/opinion/criminal-
justice/3590085-securing-public-safety-without-mass-
incarceration-or-deepening-racial-injustice/

Families Against Mandatory Minimums. (n.d.). *Alternatives to incarceration in a nutshell.* FAMM. https://famm.org/wp-content/uploads/FS-Alternatives-in-a-Nutshell.pdf

Fischer, B. (1995). Drugs, communities, and "harm reduction" in Germany: The new relevance of "public health" principles in local responses. *Journal of Public Health Policy, 16*(4), 389-411. https://doi.org/10.2307/3342618

Franklin, D. (2021, June 9). *Poll results on American attitudes toward War on Drugs.* ACLU. https://www.aclu.org/other/poll-results-american-attitudes-toward-war-drugs

Goncalves, R., Lourenco, A., & Nogueira da Silva, S. (2015). A social cost perspective in the wake of Portuguese strategy for the fight against drugs. *International Journal of Drug Policy, 26*(2), 199-209. https://doi-org.ezproxy.lib.utah.edu/10.1016/j.drugpo.2014.08.017

Henrichson, C., Rinaldi, J., & Delaney, R. (2015, May). *The price of jails: Measuring the taxpayer cost of local incarceration.* Vera. https://www.vera.org/publications/the-price-of-jails-measuring-the-taxpayer-cost-of-local-incarceration

Johnson, A., Ali-Smith, M., & McCann, S. (2022). *Diversion programs are a smart, sustainable investment in public.* Vera Institute of Justice. https://www.vera.org/news/diversion-programs-are-a-smart-sustainable-investment-in-public-safety

Karp, D. R., Bazemore, G., & Chesire, J. D. (2004). The role and attitudes of restorative board members: A case study of volunteers in community justice. *Crime & Delinquency, 50*(4),

487-515.   https://doi-org.ezproxy.lib.utah.edu/10.1177/0011128703260262

Kubic, M. W., & Pendergrass, T. (2022, August 29). *Diversion programs are cheaper and more effective than incarceration. Prosecutors should embrace them.* ACLU. https://www.aclu.org/news/smart-justice/diversion-programs-are-cheaper-and-more-effective-incarceration-prosecutors

Lowry, M., & Kerodal, A. (2019). *Prosecutor-led diversion.* Center   for   Court   Innovation. https://www.courtinnovation.org/sites/default/files/media/document/2019/prosecutor-led_diversion.pdf

Luciano, A., Belstock, J., Per Malmberg, G.A., McHugo, G. J., Drake, R. E., Xie, H., Essock, S. M., & Covell, N. H. (2014). Predictors of incarceration among urban adults with co-occurring severe mental illness and a substance use disorder. *Psychiatric   Services,   65*(11),   1335-1331.   https://doi-org.ezproxy.lib.utah.edu/10.1176/appi.ps.201300408

Marion, N. A. (2002, December 4). *Community saving in Ohio cost savings and program effectiveness.* Justice Policy Institute. https://www.prisonpolicy.org/scans/jpi/cc_ohio.pdf

Martinez, R. (n.d.). *Exploring Oregon's measure 110 and the future of drug reform policy reform.* Psychedelics Today. https://www.psychedelicstoday.com/2021/05/12/exploring-oregons-measure-110-and-the-future-of-drug-policy-reform/#:~:text=According%20to%20analysts%2C%20Measure%20110%20is%20slated%20to%3A,%2448.6%20million%20from%20ending%20arrests%2C%20jailings%2C%20and%20convictions.

Mauer, M. (2009, April). *The changing racial dynamic of the War on Drugs.* The Sentencing Project. https://www.jstor.org/stable/resrep27341

Mauer, M., & King, R. S. (2007). *A 25-Year*

quagmire: The War on Drugs and its impact on American society. The Sentencing Project. https://www.sentencingproject.org/publications/a-25-year-quagmire-the-war-on-drugs-and-its-impact-on-american-society/

McDonald, D. C. (1988). *Restitution and community service.* Office of Justice Programs. https://www.ojp.gov/ncjrs/virtual-library/abstracts/restitution-and-community-service#additional-details-0

Miron, J. A., & Waldock, K. (2010). *The budgetary impact of ending drug prohibition.* Cato Institute. https://www.cato.org/sites/cato.org/files/pubs/pdf/DrugProhibitionWP.pdf

Mississippi Department of Corrections. (2021). *Annual report 2021.* https://www.mdoc.ms.gov/Admin-Finance/Documents/2021%20Annual%20Report.pdf

Mueller-Smith, M., & Schnepel, K. (2018). *Diversion in the criminal justice system.* https://sites.lsa.umich.edu/mgms/wp-content/uploads/sites/283/2018/08/Diversion.pdf

National Center for Drug Abuse Statistics. (n.d.). *Drug related crime statistics.* NCDAS. https://drugabusestatistics.org/drug-related-crime-statistics/

NCSL. (2017). *Pretrial diversion.* National Conference of State Legislators. https://www.ncsl.org/research/civil-and-criminal-justice/pretrial-diversion.aspx

Nelis, A. (2021). *The color of justice: Racial and ethnic disparity in state prisons.* The Sentencing Project. p. 6 https://www.sentencingproject.org/wp-content/uploads/2016/06/The-Color-of-Justice-Racial-and-Ethnic-Disparity-in-State-Prisons.pdf

Orsini, M. M. (2017). Frame analysis of drug

narratives in network news coverage. *Contemporary Drug Problems, 44*(3), 189-211. https://doi.org/10.1177/0091450917722817

Patchin, J. W., & Keveles, G. N. (2015, June 4). *Alternatives to incarceration: An evidence- Based research review* [Summary of findings]. Northwest Wisconsin Criminal Justice Management Conference, Cable, Wisconsin. https://people.uwec.edu/patchinj/crmj103/Alternatives%20to%20Jails%20Research%20Review%20-%20Executive%20Summary.pdf

Picard, S., Tallon, J. A., Lowry, M., & Kralstein, D. (2019, July). *Court-Ordered community service: A national perspective.* Center for Court Innovation. https://www.courtinnovation.org/sites/default/files/media/document/2019/community_service_report_11052019_0.pdf

Piquero, A. (2010, October 6). *Cost-Benefit analysis for jail alternatives and jail.* Social Value UK. https://socialvalueuk.org/

Pretrial Justice Institute. (2017). *Pretrial justice: How much does it cost?* Reimagining Justice. https://portal.ct.gov/-/media/Malloy-Archive/Reimagining-Justice/Reimagining-Justice—Pretrial-justice-at-what-cost-PJI-2017.pdf?sc_lang=en&hash=2D9ACDE29DDD4EE58364277140A64B8F

Rempel, M., Labriola, M., Hunt, P., Davis, R., Reich, W., & Cherney, S. (2018). *NIJ's multisite evaluation of prosecutor-Led diversion programs.* Office of Justice Programs. https://www.ojp.gov/pdffiles1/nij/grants/251665.pdf

Roberts, D. E. (2004). The social and moral cost of mass incarceration in African American communities. *Stanford Law Review, 65*(5), 1271-1305. https://www.jstor.org/stable/40040178

Rouhani, S., McGinty, E. E., Weicker, N. P., White, R. H., LaSalle, L., Barry, C. L., & Sherman,

S. G. (2022). Racial resentment and support for decriminalization of drug possession in the United States. *Preventive Medicine, 163*(107189), 1-8. https://doi.org/10.1016/j.ypmed.2022.107189

Sawyer, W., & Wagner, P. (2022, March 14). *Mass incarceration: The whole pie 2022.* Prison Policy Initiative. https://www.prisonpolicy.org/reports/pie2022.html#lf-fnref:14

Sutton, M. (2021, November 3). *Drug decriminalization in Oregon, one year later: Thousands of lives not ruined by possession arrests, $300 million+ in funding for services.* We are the Drug Policy Alliance. https://drugpolicy.org/press-release/2021/11/drug-decriminalization-oregon-one-year-later-thousands-lives-not-ruined

Taxy, S., Samuels, J., & Adams, W. (2015, October). *Drug offenders in federal prison: Estimates of characteristics based on linked data.* US Department of Justice. https://bjs.ojp.gov/content/pub/pdf/dofp12.pdf

The Sentencing Project. (2018, April 19). *Report to the United Nations on racial disparities in the US criminal justice system.*

https://www.sentencingproject.org/publications/ un-report-
on-racial-disparities/

The Sentencing Project. (2021, May 17). *Trends in US corrections.* https://www.sentencingproject.org/publications/ trends-in-u-s-corrections/

US Department of Justice. (2020, January 29). *9-22.000 – Pretrial diversion program.* https://www.justice.gov/jm/ jm-9-22000-pretrial-
diversion-program#:~:text=Pretrial%20diversion%20(PTD)%20is%20an,at%20the%20pre%2Dcharge%20st age

Vera Institute on Justice. (n.d.). *Ending mass incarceration.* Vera. https://www.vera.org/ ending-mass-incarceration

Viars, S. (2016). *Economic implications of decriminalization* (Publication No. 10249060). [Master's thesis, University of North Carolina at Charlotte]. ProQuest Dissertation Publishing.

Wilderman, C., & Wang, E. A. (2017). Mass incarceration, public health, and widening inequality in the USA. *The Lancet, 389*(10077), 1464-1474. https://doi.org/10.1016/ S0140-6736(17)30259-3

Wood, W. R. (2013). Soliciting community involvement and support for restorative justice through community service. *Criminal Justice Policy Review, 26*(2), 135-155. https://doi- org.ezproxy.lib.utah.edu/10.1177/0887403413499580

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SECTION XIV

Honors

148. **The Great Salt Lake and the Bear River in Crisis: A Holistic Analysis**

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ABSTRACT

Bear River and Great Salt Lake are two ecological systems currently in the public eye due to a shrinking Great Salt Lake, and Bear River being its largest tributary. This paper attempts to explore a variety of topics in and around the lake and basin, to see how everything is currently being, or not being managed, and this paper argues that a holistic view of the lake and river are needed in order to resolve the current ecological crisis.

INTRODUCTION

Great Salt Lake is currently at its lowest level in recorded history.1,2 This is not just due to climate change or to the current drought, but also to woeful shortsightedness. Great Salt Lake is a terminal lake in Utah, USA, and the largest saltwater lake in the western hemisphere. Its' main tributary and area of chief concern is Bear River, which provides 58% of the stream flows received by Great Salt Lake yearly.3 Average yearly stream flows from Bear River to Great Salt Lake have not changed in recorded history, so this must mean the decline in lake elevation is caused by some outside factor.4 Problems caused by human activities within a definable geographical area can be solved, and to do so a crucial step to reframe our thinking about Great Salt Lake and Bear River. In this paper, I will argue that viewing and treating Bear River Watershed and Great Salt Lake holistically is beneficial because it will protect the health of all the interrelated and reliant ecosystems, and protect the health of humans living in and around these ecosystems. Most importantly, incorporating the socio-political history of the Northwestern Shoshone tribes who lived here and the settlers that expelled them let us acknowledge and begin to repair some of the wrongs done to the Shoshone people.

Firstly, to prove to that there is a crisis and that it is anthropogenically caused, we begin with the history of Bear River stream flows. Ongoing data

published by the USGS (United States Geological Survey) reveals the consistency of water resources within Bear River watershed, and the need for holistic solutions to our water crisis. The research shows that over the past 80 years, the average yearly streamflow through Bear River headwaters has stayed consistent, showing no change due to drought or wet years, historically.5 These findings show that the crisis of drought and shrinking water level in Great Salt Lake are not entirely caused by the historic drought we are living through, but instead an artificial crisis.

The Bear River and the Great Sale Lake are intimately connected, with Bear River responsible for 58% of the water flowing into Great Salt Lake yearly. The other two tributaries, the Jordan and the Weber, are responsible for the rest. Since Bear River is so important for the health of Great Salt Lake, its' (Bear River's) health and maintenance are reflected in the health and maintenance of Great Salt Lake. And currently, Great Salt Lake is experiencing a historic drought, with water levels the lowest they have been in over 100 years. And yet, the water levels in the headwaters of Bear River remain the same, so there must be some other explanation for the current shrinkage of Great Salt Lake than the current narrative of drought and lack of water. 6 Ongoing data taken by the USGS at Bear River near its terminus into Great Salt Lake points to anthropogenic effects on river flows within Bear

River watershed. The data shows that there is a slight decreasing trend of stream flows at the Lower Bear River, however, statically analysis of the data implies that this trend is very weak. This evidence is compelling, considering anthropogenic effects on Bear River basin.

Between its headwaters and its terminus, the flows from Bear River do increase by virtue of running down a large watershed. Despite this, the total amount of water that flows out of Bear River basin has been decreasing, despite evidence that precipitation in its headwaters has been staying consistent. This means that human effects, mostly agricultural water uses, have been effective at decreasing the total streamflow of Bear River and thus the water level of Great Salt Lake.

DISCUSSION

In the recently published book Great Salt Lake Biology there is a chapter on environmental sociology, and the specific intersection between humans (post-colonization humans) and Great Salt Lake, a relatively unexplored niche in the history of Great Salt Lake. 7 This chapter provides valuable insights on the sociopolitical reality of how people living in the Salt Lake valley interact with and think about Great Salt Lake, and how that effects Great Salt Lake and, in turn, affects us once again. The research shows that those people who live near the lake and in the valley hold incredibly mixed opinions, with a trend towards realizing the lake as

a negative thing. As the author references from the Utah Historical Quarterly "There's a sturdy cliché about the Great Salt Lake: most people think the lake is too much. Too flat, too shallow, too salty, too stinky to love—or even to refrain from fouling."8 Much of this public perception is created in part due to the cultural narrative (and self-fulfilling prophecy) and the human effects on the lake. This research implies the need for a new narrative around the lake in order to spearhead a movement to save it.

Funnily enough, a lot of the negative aspects people associate with Great Salt Lake, the smell, the flies, the dust, are exacerbated when the water elevation is low, and are abated when the elevation is high.9 When Great Salt Lake is seen as negative in the collective unconscious, we tend to care less about it, to give less energy to it, which in turn makes those problems worse. The first time anyone cared enough to study the lake in any detail was when the brine shrimp industry was on the brink of collapse. When Great Salt Lake is doing poorly, that's when we ought to care about it the most. The issue of public perception is a huge deal and one that is shaped in large part by the mass media and the state, which have their own interests at heart (see pray for rain, below). Fixing the public perception of the lake could be seen one part of a holistic view of repairing Great Salt Lake.

Extremely   recent   research   published   in

September of 2021 by the Journal of Geophysical Research highlights some extremely alarming trends in groundwater loss and associated crust-deformation in the area within and around Great Salt Lake.10 The research found that the amount of crustal deformation alarmingly reveals a much greater loss of surrounding groundwater, up to 48 km3 of groundwater.11 These findings shock us into considering how Great Salt Lake can be an indicator for many more nested problems in the surrounding area, some of which are invisible to the naked eye.

Forty-eight km3 of groundwater doesn't get up and walk away. This means that the pumping of groundwater has exceeded the recharge rate of Great Salt Lake tributaries to an extreme amount. Along with surface water exploitation, i.e., the creation of canals and dams, groundwater exploitation is a problem more pervasive and much less visible. Groundwater overexploitation lowers the water table, which makes it harder to return surface water elevation to its previous level because it needs to recharge the water table first. It also has deleterious effects on nearby plants, depriving them of water they previously had access to. This causes the ground to dry up, being more vulnerable to large seismic events. Notably, in this paper the authors find that seismic events happen more during dry years. This entire process is dubbed an externality, and is much less regulated then surface water within the court of law. You can pump groundwater until the well runs dry, and the fact that will cause untold environmental, economic, and human health damage is acceptable to the status quo.

These data also highlight some extremely

alarming trends in groundwater loss and associated crust-deformation in the area within and around Great Salt Lake. The research found that due to surface and ground water loss, the crust of the Earth around the entirety of Great Salt Lake has risen up to six mm.12 This finding and its related affects are unknown to us at the present moment, highlighting a need for further research on the effect of crust deformation.

The researchers used GPS data and ground surface imaging programs to analyze how the crust of the Earth itself has responded to a shrinking GSL. Water has weight. A lot of water has enough weight to deform the crust of the Earth so much that its visible on a satellite. Since Great Salt Lake is so expansive, fluctuation in its water level affect the crust of the Earth to an impressive amount. Without meaning to, we've raised up the Earth 6mm. Moving an incredible amount of Earth by overusing the surrounding water resources. The consequences of these actions are understudied and require more investigation. Suffice to say that due to the interconnected nature of all things, the surface of Great Salt Lake shrinking results in a series of problems, all interlinked and coinciding, some of which are obvious to us than others.

Research published in the Journal of Ecological Restoration taking place at the Columbia River tests the effects of artificially recreating wetland conditions, and how an environment can heal with

the help of humans. In certain colonized agricultural lands along the estuary of the Columbia river, several logs were artificially placed with the intention of recreating the historical forced-step pool morphology of the estuary before colonization. 13 The resulting beneficial effects on salmon and water chemical composition urge us to consider how we can effectively restore extensible modified biomes to create a more robust and resilient ecosystem.

The GSL and its tributaries are responsible for one of the largest wetlands in any desert. This wetland has been subject to much exploitation and land use change due to the colonizing and the ever-expanding process of capitalism. Currently, there is both a prison and an inland port being built upon Great Salt Lake wetland, the philosophical implications of which are outside of the scope of this paper. The more tangible process going on is that the land being destroyed. The ground is drying up and the biome created by the trees and the grasses and soil is being destroyed, resulting in a drier climate and the inability to retain water. Negative things are being done to the land, which result in negative consequences. This paper shows, however, that they can be restored and healed. Featureless stretches of fallow farmland can be restored to healthy marshlands, swamps and estuaries.

This research also took into account the local

and preexisting beaver dams that were within the scope of lands they were restoring. Evidence of cohabitation of juvenile salmon within beaver dams and their associated pools highlights how ecosystem processes of one animal can be beneficial or even necessary for another.14 These findings want us to consider how and why an environment acts in the way that it does, in a more holistic way. It would be unfair to try and talk about the restoration of salmon habitats without mentioning the boons that beavers provide to salmon.

So far, this paper has only explored the science side of viewing this entire picture holistically. Just as important is exploring the socio-political and economic history of Bear River watershed. Historically, the Northwestern Shoshone tribe has lived upon this land, until 1863. This history is barely acknowledged within our discussions about the Great Salt Lake, yet it incredibly crucial to discuss if we want to have a chance at fixing the problems we have caused. On January 29, 1863, the Bear River Massacre was done to the Shoshone people by the California Volunteer Infantry.15 The purpose of the massacre was "to make the roads and trails of Utah Territory secure to travelers." The history of this place reveals the horrifying legacy of settler colonialism within Utah and the Bear River watershed specifically, and how these historic injustices must be approached and redressed to

have Great Salt Lake and Bear River stay alive and whole.

If we are to view an ecosystem holistically and concede that the human presence has an effect on the river and the lake, we need to address the historical human presence in and around Bear River. One of the most important historical events to happen in the Cache valley was Bear River Massacre. It was a purposeful stealing of land and murdering of people to open up an area for exploitation, and the descendants of the people who stole that land still live there. Settler colonialism and the historic injustices have never went away, the legacy and sociopolitical consequences of those actions are still affecting us, even if a small parcel of land is now owned by the Shoshone people and deemed a historic site.16 The legacy of settler colonialism can be seen in the elevation of Great Salt Lake.

This legacy can be seen not just in historically low levels, but in historically high ones as well. Taking the historic high of 4,211.85 feet in 1987, which was deemed a crisis by the governor at the time.17 This is a topic Terry Tempest Williams explores in her book, Refuge. We can see the presence of Lake Bonneville, Great Salt Lake's ancestor all around us, the Shoreline Trail around the Salt Lake Valley, the notches carved into mountains as the lake carved its huge and winding path. A historic flooding event like the one in

1986-7 could have been prepared for in some way, though such methods are outside of the scope of this thesis. This is to say, the way the Salt Lake Valley was settled and transformed left the state completely unable to rapidly and meaningfully respond to the crisis. As Williams writes:

> "What would happen," I asked, "if the governor said, 'I've decided to do nothing. Great Salt Lake is cyclic. This is a natural phenomenon. Our roads are built on a flood plain. We will move them.' " I looked at my father. "He'd be impeached," Dad said, laughing. "The lakeshore industry is hurting financially. The pumping project is a way to bailout the salt and mineral companies, Southern Pacific Railroad, and a political career as well."18

The west desert pumping station is one more marker of the presence around Great Salt Lake. Erected in 1986, it was the states effort to control Great Salt Lake, rather than let it do what it has done for time immemorial. The lake cannot do what it has done, because the economy is built on the basis of denying the reality of Great Salt Lake. It cannot be cyclic, wild or real, lest industry suffer. On the one hand, we see how the current settler-colonist regime handles this historic high, and on the other, we have the words of Darren Parry, in his lecture Voices from Dust: A Shoshone Perspective

on the Bear River Massacre. "We traveled to different areas when the game was plentiful and the berries and seeds were abundant." If somewhere wasn't a nice place to live, like a flood, they would get up and leave. An ability and perspective that has been totally lost today. If there's too much water in Great Salt Lake, pump it out, if there's not enough water, ship it in from the Pacific Ocean.19

This is a nested issue, water rights in Utah. Due to the prior appropriation doctrine, leaving water in Bear River is simply not an option. All water rights have been divvied up for Bear River, and in order for a given consumer to maintain their water right, they must prove the water is being put to "productive use."20 In the letter of the law, this means some sort of consumptive use, like agriculture or a municipality.21 Leaving the water in the river, if you had a water right, would mean your right is forfeit. Putting water towards agricultural use does not consume all of the used water, some of it percolates back into the ground and is returned to the river. According the Statewide 2021 Water-Related Land Use Inventories in Utah, published by the Utah Department of Natural Resources, all agricultural land along the length of Bear River is farmed using irrigation (sprinkler/flood) methods.22 The Statewide 2021 Water-Related Land Use Inventories in Utah also includes a detailed listing of every single parcel of agricultural land, and what it is used for. Along

Bear River drainage basin, this is broken down into >1,900,000 acres, and >95,000 individual parcels counted.23 Alfalfa, comprising 291,645 acres and 28,370 individual parcels, is the most popular agricultural use, and will be further explored below.24

Bear River basin streamflow is 799806.55 acre-feet.25,26 Using the diversion calculated from Lozada's report, alfalfa production diverts 2.12 acre-feet of water per acre harvested.27 Of note, agricultural use is not 100% consumptive of water diverted, in fact it diverts a great amount more than 2.12 feet of water per acre, around five feet of water per acre, due to inefficiencies in growing and irrigation. The 2.12 number is the theoretical minimum amount of water alfalfa consumes in the growing process, which is entirely consumed. This, in Bear River basin, accounts for 618,287.4177 acre-feet of water diverted for alfalfa production, just in 2020. Since Bear River basin flows through and is diverted by three different states, there is no clean estimate for the revenue produced by this region. We can however, take other numbers to provide an estimate of the value produced. In 2020, the National Agriculture Statistics Service (NASS) reported that the average yield of alfalfa in Utah was 4.1 tons/acre, with a revenue of $298/ton.28 Thus, the total value of all alfalfa grown in Bear River basin in 2020 was $337,211,624.5. Including manufacturing costs at a rate of 45% of total

revenue lowers total profit generated by alfalfa farming to $185,466,393.5.29 One would assume farmers pay for the water used in production, but certain legal stipulations mean that farmers on the whole do not pay for the water used in production.30, 31 One acre-foot of water thus produces $299. If farmers were charged $0.00091 per gallon of water used, alfalfa farming becomes unprofitable. As a token example, the cheapest price of residential water is $0.0022 per gallon. If farmers were charged equal to the cheapest residential user, alfalfa warming would become wildly unprofitable. If the alfalfa farmers were compensated to do nothing at the same rate, just leave the water in the river, it would cost every Utahn $34.32 To subsidize the entire profit generated by all hay farming Bear River Basin, it would cost every Utahn $88.33.

This is to imply that the crops grown in Bear River Basin are not integral or otherwise a key component of the food system of Utah, and that their non-production would not be adversely detrimental. If they were, then despite the clear financial/environmental/health incentives to not grow these crops, we would be forced to grow them anyway, in order to prevent a crisis in feeding the population. If that were the case, the best-case scenario I could argue for would be to conserve and minimize water consumption, rather than the case of not use it at all. Regardless, the crops grown in

Bear River Valley are not integral to our system because a large percentage of them are exported, and the tremendous amount of food waste inherent in the system.

One, the fact that crops are exported. This is fact is plainly evident. All of the crops that are exported elsewhere represent only profit to the farmers, and are not integral to Utah's food system. In 2020, 29% of all Utah hay was exported.34 This percentage represents exclusively turning water into cash, by shipping it out of state. This percentage entirely could be safely removed without worrying about long-term effects to Utah's food system, and would bolster Great Salt Lake by a charitable 0.44 feet, or about five inches.35 Two, the inherent amount of food waste in the system already. As hay is used as livestock feed, not as a direct to consumer good, it is difficult to measure precisely how much of it is wasted in the food system. A 2014 estimate from the USDA finds that 28% of meat products end up unconsumed as waste.36 Assuming the remaining 71% of non-exported hay goes towards feeding livestock, that accounts for 19.8% of the total hay grown in Bear River Valley ends up unconsumed, as waste. This would raise Great Salt Lake by 0.28 feet, or about three and a half inches. This, together accounts for 48.8% of the hay grown in Bear River Basin being entirely irrelevant to the healthy and proper functioning of our food system. Just these two facts alone account for almost half of the hay

grown, and this percentage could be reduced further by converting the hay to a crop eaten by people, to reduce the inherent waste in trophic levels, or various other means of more efficient agriculture.

   This, to be clear, is not an issue to be hoisted directly onto agri-business, As NASS reports that 95% of farms in Utah are family farms, which are defined as "any farm where the majority of the business is owned by the producer and individuals related to the producer."37 This is a cultural and historical problem, tracing its origins back to the settlement of the American West, and for Bear River, the Bear River Massacre on 1863. Land was stolen and settled by individual families, and those families on the whole, have not gone anywhere in the proceeding 160 years. The Northwestern Shoshone haven't gone anywhere, either. There is obvious tension here, and one that exists outside of numbers and lake elevation. This holistic view includes something, that on the whole, Utahns have forgotten. Take one of the two plaques erected at the Bear River Massacre Site, the one from 1932 reading:

> The Battle of Bear River was fought in this vicinity January 29, 1863. Col P. E. Connor, leading 300 California Volunteers from Camp Douglas, Utah, against Bannock and Shoshone Indians guilty of hostile attacks

on emigrants and settlers, engaged about
500 Indians of whom 250 to 300 were killed
or incapacitated, including about 90
combatant women and children. 14 soldiers
were killed, 4 officers and 49 men wounded,
of whom 1 officer and 7 men died later. 79
were severely frozen. Chiefs Bear Hunter,
Sagwitch, and Lehi were reported killed.
175 horses and much stolen property were
recovered. 70 lodges were burned. —
Franklin County Chapter, Daughters of the
Utah Pioneers, Cache Valley Council, Boy
Scouts of America and Utah Pioneer Trails
and Landmarks Association.

This is the history that farmers in Bear River have chosen to remember. Combatant women and children. This is the history that has given us a drying lake and a governor telling us to pray for rain. Not only do the legal and economic aspects of water in Utah need a systemic change, the culture and lens with which we view the history, legacy, and responsibility of those living there today need one as well. Let us not forget that ten years after the massacre, a Mormon missionary baptized the 102 members of the Northwestern Shoshone in Bear River, an incredibly potent metaphor for the white-washing, obfuscation and forgetfulness of history under the Mormon regime. There is injustice here that needs rectifying.

So far, I've been showing examples of how things

are related, the cause and effect between two things. Sometimes it's obvious, like the case of beaver dams being nice places for fish to live in, and sometimes it's more subtle, like the overexploitation of groundwater leading to the crust elevating and earthquakes happening more.38,39 All of these things aren't just related to things I've described, they are related to everything else. A system as infinitely complex as a river and its terminus is going to have a thousand cascading effects on all of things it passes by and flows over, and all of those things are going to affect the river right back. By analyzing all of them, together, we get a clearer picture of how little we understand and how extensive our actions are. This is a holistic view of Great Salt Lake. We must emphasize the importance of the whole and the butterfly effect one thing can have on everything else. This process of holistic analyzing doesn't end with our economy and the environment, (e.g., how does building the inland port effect the surrounding water table? What about mosquito populations?) it considers history and ongoing socio-political process. The Massacre at Bear River and the areas' subsequent colonization can be seen in the water elevation of Great Salt Lake. Will our governor address that and try to remedy it? No, the best he can do is tell us to pray for rain.40

The fact that viewing this GSL and Bear River systems holistically would be a great boon and a

potentially politically radical shift in thinking can be seen as the thesis, and the antithesis is the fact that institutions of power currently rely on the negation of that information to continue over-exploiting the water and land to great economic benefit. A synthesis of this dialectic could be this: Overhauling the current GSL managing institutions within the government to instead be both co-managed by the government of the Northwestern Shoshone, and expanded from just Great Salt Lake to the entire Bear River watershed. It has to be managed for protection, and as little use as possible. There is some inkling of this idea already being seen in Bear River. There is Bear River Migratory Bird Refuge, which is a protected and undeveloped area of 299 km.41. The Northwestern Shoshone purchased Bear River Massacre site, in 2005. Then, currently are fundraising and planning efforts to both build the Boa Ogoi (Wuda Ogwa) Cultural Interpretive Center.42 A meaningful improvement on the two lackluster plaques erected there already, and one that won't be erasing history. In addition, the Northwestern Shoshone have begun working with Utah State University to restore the site to how it looked in 1863.43 This is a fascinating concept, very much in line with a holistic overview of Bear River. Tying ecology into history, creating a historical document in the plants and animals living in Bear River Basin, could and should be the start of a more robust analysis of the entire

watershed, and one that synthesizes everything discussed here.

A potent combination of both of these ways of protecting the land, native ownership and US government exercising power, can result in tangible protecting for the water and the land. And hopefully one that will be seen soon in the Boa Ogoi Cultural Interpretive Center. This idea quite obviously steps on the toes of the farmers and agribusiness rewidening there, but as it stands, they will kill us all if they continue to rule over the hydro-geologic regime unchecked.44 None of the water from Bear River even reached Great Salt Lake in 2020 for four months.45 Every single drop got appropriated. If none of the blood reached your brain for four months, you would be dead. The best time to act was in 1863, the second-best time is now. Go see the lake, agitate for change, destroy a combine harvester, give all of your money to the Shoshone, whose land you are currently living upon.46

CONCLUSION

In sum, treating and managing Bear River Watershed and Great Salt Lake holistically is beneficial because it will protect the health of all the interrelated and reliant ecosystems; and protect the health of humans living in and around these ecosystems. Most importantly, incorporating the socio-political history of the Northwestern Shoshone tribes who lived here and the settlers

that expelled them let us acknowledge and begin to repair some of the wrongs done to the Shoshone people whose land we are living upon and that I am writing about. Of all the problems currently being faced in society, this one we can actually stand up to and fix in a speedy and timely manner. We can't pull CO2 out of the atmosphere and solve climate change in a day, but we could sign over land rights back to the Shoshone, and Great Salt Lake could be filled back up before we know it, increasing snowfall, reducing asthma and making our world a richer and more robust place to live. If we don't, we will all have to live on arid, dead land, chocking on arsenic laced dust and praying for rain in January.

Bibliography

 "2017 Census of Agriculture, State Profile, Utah," National Agricultural Statistics Service,
(2017).

"2022 STATE AGRICULTURE OVERVIEW." National Agricultural Statistics Service, (2022).

Bears, Edwin, "National Register of Historic Places Registration Form, Bear River Massacre"
United States Department of the Interior, National Park Service, (1990), 13.

"Bear River Migratory Bird Refuge." U.S. Fish and Wildlife Service, accessed December 3,
2021.   https://www.fws.gov/refuge/
bear_river_migratory_bird_refuge/

"Bear River Near Corinne, UT." USGS, 2022. Last accessed April 20, 2023.
https://bearriverinfo.org/watershed-data/USGS-streamflow-data

 Brian Maffly, "Rescue Great Salt Lake with seawater from the Pacific? Utah lawmakers
consider it." Salt Lake Tribune, January, 2022.

Buzby, Jean, Wells, Hodan, and Hyman, Jeffrey, "The Estimated Amount, Value, and Calories
of Postharvest Food Losses at the Retail and Consumer Levels in the United States." United States Department of Agriculture, (2014) 12.

CMN Staff, "Tribe Remembers Nation's Largest Massacre," Indian Country Today, March
2008,   https://web.archive.org/web/20170911070021/
https://indiancountrymedianetwork.com/news/tribe-remembers-nations-largest-massacre-2/

Cox, Spencer, "A Weekend of Prayer." the Governor's Office of the State of Utah, June 3,
2021.   1:26.   https://www.youtube.com/watch?v=0A2KkyDGHRY&t=1s

 Cox, Spencer, "Proclamation 2022-01." the Governor's Office of the State of Utah, November
3rd, 2022.

Diefenderfer, H.L. and Montgomery, D.R., "Pool Spacing, Channel Morphology, and the
Restoration of Tidal Forested Wetlands of the Columbia River, U.S.A." (Journal of Restoration Ecology, 2009), 158.

Gehrke, Robert, "The Great Salt Lake is disappearing. If we don't fix it, we'll pay the cost with
our health and economy, Robert Gehrke says." Salt Lake Tribune, November 4, 2021. Accessed from https://www.sltrib.com/news/environment/2021/11/22/great-salt-lake-is/

"Great Salt Lake Reaches New Historic Low." Communication and Publishing, USGS, 2021.

Hockaday and Ormerod, K.J., "Western Water Law: Understanding the Doctrine of Prior
Appropriation." University of Nevada, Reno, 2020.

Lozada, Gabriel, "Agricultural Water Use, Hay, and Utah's Water Future." University of Utah
Economics Department, (2021).

Madsen, Marley, "Boa Ogoi: Restoring Sacred Land 150 years after the Bear River Massacre."
Utah State Today, 2020.

Null, Sarah. And Wurtsbaugh, Wayne, "Water Development, Consumptive Water Uses, and
Great Salt Lake." in Great Salt Lake Biology, Springer, (2020), 5.

"Statewide 2021 Water-Related Land Use Inventories in Utah." Utah Department of Natural
Resources, (2021).

Trentelman, Carla. "Relationships Between Humans and Great Salt Lake: Dynamics of Change."
in Great Salt Lake Biology, Springer, (2020), 61.

"USGS Real-Time Streamflow Data," Bear River Watershed Information System, accessed
November 20th, 2021, https://bearriverinfo.org/watershed-data/USGS-streamflow-data

Williams, Terry Tempest, Refuge. Canada: Vintage Books, 2018, 239.

Young, Zachary M., Kreemer, Corné, and Blewitt, Geoffrey, "GPS Constraints on Drought-
Induced Groundwater Loss Around Great Salt Lake, Utah, With Implications for Seismicity Modulation." Journal of Geophysical Research, (2021).

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