## RANGE: Undergraduate Research Journal

# RANGE: Undergraduate Research Journal

### VOLUME 23, ISSUE 2 (2022)

ANNIE ISABEL FUKUSHIMA (EDITOR IN CHIEF) University of utah • Salt Lake City



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# An Introduction - Launching Range: Undergraduate Research Journal

#### Annie Isabel Fukushima (Editor in Chief)

The Office of Undergraduate Research (OUR) journal was launched in 2001, publishing student abstracts every year. Bound publications may be found archived in the J. Willard Marriott digital library and hard copies of bound books sit archived in the Sill Center OUR offices. In 2015, the journal went one hundred percent on-line on the OUR website. Central to the University of Utah mission statement is generating and sharing new knowledge, discoveries, and innovations to engage local and global communities to promote education, health, and quality of life. Collaborations with and the support of undergraduate research is central to these endeavors. Therefore, with a will to create greater access in student and faculty collaborative publications, the Office of Undergraduate Research hired Eliana Massey to serve as the OUR Publications and Public Research to work with leadership to figure out: what would the new face of the undergraduate research journal

look like? Could we find a new home for the journal articles published with the journal? Could the journal be both accessible online, archivable, searchable, and indexed? After meeting with Anne Morrow at the Marriott Library, I am pleased to announce that OUR is launching the undergraduate research journal on a new platform: the Utah Education Network with PressBooks. The platform makes the journal searchable, more easy to navigate, and accessible on an online platform. The platform represents the role of the universities in making available the public cutting edge research.

After considering multiple platforms, a new home for early career scholars in undergraduate research at the University of Utah, I am thrilled to share with our readers and contributors that to signal the new location of the journal with Press Books the journal is premiering with a new name to celebrate this transformation, Range: Undergraduate Research Journal. There is much significance in a new name. To arrive at a new name, students were surveyed, ideas gathered from student-peer discussions with the staff of the Office of Undergraduate Research (Eli Covarrubias, Cindy Greaves, Shelly Parker, Jude Ruelas, and Megan Shannahan and myself). After deep thought and reflection, we collectively arrived at the name "Range." When considering the diversity of contributions representing 18 colleges at the University of Utah, the term "range" surely is suitable to describe what is found in this undergraduate research journal. Range represents the breadth of contributions found in the issues submitted and published every year. The depth in scope of scholarly contributions that are found in the journal represent the multitude of points of views, methodologies, life experiences, positionalities, disciplines, and research contributions published every year with OUR. Here Eliana, undergraduate research leader and the launch issue

student editor, provides her own perspective on the concept of range as playing on an intellectual contribution in addition to pointing to the geographical location of University of Utah:

> "Likely as a result of tectonic extension that began around 17 million years ago, much of the inland Western United States and northwestern Mexico is characterized by abrupt alternation between mountain chains and arid valleys. This physiographic region is referred to as the Basin and Range Province. Within the Basin and Range Province, Salt Lake City, the home of the University of Utah, lies next to the Wasatch Range which stretches approximately 160 miles. Undergraduate students at the University of Utah, like the region in which they live and study, extend far and wide–in terms of their interest and impact. We chose the name for this journal as a way to honor the bioregion we live in as well as the diversity of research that our students engage in."

Range invites readers to remember the importance of context and place for research. Recognizing that the University of Utah land, which is named for the Ute Tribe, is the traditional and ancestral homeland of the Shoshone, Paiute, Goshute, and Ute Tribes the journal also affirms that the University of Utah recognizes and respects the enduring relationship that exists between many Indigenous Peoples and their traditional homelands. We respect the sovereign relationship between tribes, states, and the federal government, and we affirm the University of Utah's commitment to a partnership with Native Nations and Urban Indian communities through research, education, and community outreach activities. To nourish our communities, is to feed the mind. We hope that this journal in its new publication form, newly named as Range: Undergraduate Research Journal, may serve as a new

intellectual home for undergraduate researchers whose curiosities take them into research across the disciplines, traversing the steeps in intellectual thought in their own fields, as they are connected to other fields of inquiry.

Readers of *Range: Undergraduate Research Journal* will find abstracts, full length articles, creative contributions, and undergraduate student reflections about the research process. As our readers peruse the new features of the journal, we hope that readers will also appreciate a new component in the journal: reflections on undergraduate research experiences. In addition to research publications and abstracts, contributors provide brief reflections on their research experiences. Reflecting on research is a vital part of research practice. Through the diversity of contributions and reflections about research, the Office hopes that students, faculty, and staff alike, may find connection, community, and an intellectual home in the journal that welcomes student research publications as part of a central aspect of an R1 institution commitment.

It is with this I close with a deep appreciation to our contributors, mentors, Eliana Massey (Student Editor), Eli Covarrubias (Managing Editor), Cindy Greaves (Managing Editor), OUR leaders, and the OUR team (Jude Ruelas, Shelly Parker, and Megan Shannahan) who have provided important support to launch the the 2022 *Range: Undergraduate Research Journal*, Volume 23, Issue 2 (2022), formerly known as the *Undergraduate Research Journal*.

Annie Isabel Fukushima Associate Dean, Undergraduate Studies Director, Office of Undergraduate Research University of Utah

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About the Author

Annie Fukushima UNIVERSITY OF UTAH

**DEPARTMENT I** 

# College of Architecture & Planning

### 1. Why they Stay:

### **Puerto Rican**

### **Businesses in the**

#### **Flood Zone**

Olivia Vielstich (University of Utah)

Faculty Mentor: Ivis Garcia Zambrana (City & Metropolitan Planning, University of Utah)

In 2017 Hurricane Maria, a category five hurricane, hit Puerto Rico. The destruction caused by Maria is still felt around the island to this day. Many people continue to live in areas that are at high risk of future disasters. Resources that offer to move residents to more safe areas exist, but many people choose to stay in the danger zone. This project focuses specifically on businesses that chose to continue operating within the 100-year flood

zone as established by FEMA. Through 125 inperson surveys, 75 in Loíza and 75 in Comerío, Puerto Rico, we sought to understand why they stay. Our findings showed that residents of these communities were heavily influenced by the interdependencies they had with residents and other local businesses. Simply moving to another would disrupt these interdependent area relationships and could be harmful to their livelihoods or communities. For many businesses, the risk of staying in a high-risk disaster area is lower than the risk of starting over in a new place. More research can be done to understand how organizations offering assistance can make their resources more accessible to residents in Loíza and Comerío, as well as tailor assistance to better suit the wants and needs of those who are seeking aid.

About the Author

Olivia Vielstich UNIVERSITY OF UTAH

**DEPARTMENT II** 

# School for Cultural & Social Transformation

# 2. Reflection on Undergraduate Research Experience -Liuchen (Tony)

### Chen

Tony (Liuchen) Chen (University of Utah)

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

I worked with Dr. Fukushima in state-wide research on domestic violence, sexual violence, and human trafficking among Asian communities; this study led by the Gender-Based Violence Consortium explored survivor and community needs to reduce violence. I assisted Dr. Fukushima with the creation and updating of a roster or list of organizations to reach out to share information about the study among Asian immigrants and Asian diasporic communities in Utah, coordinated interviews with participants in English, taking notes, listened for patterns and themes, analyzed research results by organizing and interpreting the data, evaluating the interview transcript, attended the Undergraduate Research Education Series to enhance research skills, and presented the research project at The Virtual Fall Undergraduate Research Symposium.

Ι am majoring in Family, Community, and Human Development. After receiving my bachelor's degree, I plan to pursue a master's degree in Social Work or Human Development and later pursue Ph.D. programs. I have a deep passion for social justice to help better develop an equitable and healthy society. I am interested in participating in research, especially research related to intimate relationships, human development, and family issues. This research project involves domestic violence and deeply connects to my aim of a deeper understanding of serious relationships and family issues. I strongly believe this UROP project benefits my studies and career by enhancing my research skills and preparing me as a graduate student, and researcher.

About the Author

Tony (Liuchen) Chen (University of Utah)

3. Asian Community: Utah State-Wide Needs Assessment of Domestic Violence, Sexual Violence, and Human Trafficking Tony (Liuchen) Chen (University of Utah)

Faculty Mentor: Dr. Annie Isabel Fukushima (Division of Ethnic Studies and Sociology, University of Utah)

Domestic violence, sexual violence, and human trafficking are

prevalent issues in Utah. There are about 33.6% and 21.4% of women and men had experienced physical threats from intimate partners, respectively in Utah (Smith et al., 2017). Because of the linguistic and/or cultural barriers, few studies have explored how violence impacts Asian American or immigrant communities, which suggests that researchers should pay more attention to the Asian community to better assess the needs of victims of domestic violence, sexual violence, and human trafficking. This project is part of the state-wide needs assessment on domestic violence, sexual violence, and human trafficking that aims to explore what service needs would benefit anti-violence efforts in Utah, and mainly focus on Asian immigrant and diasporic communities in Utah. This study adopted mixed methods including quantitative methods (online survey) and qualitative methods (interview). The result shows that 1) the current legal resources for victims of violence are insufficient, 2) factors that impact insufficient legal resources include bureaucracy, unaffordable services, the rejection of services/support, the unawareness of legal services, and lack staff/providers, and 3) pressing legal needs such as divorce, custody of a child, protective/restraining order, immigration, legal needs related to domestic violence, and legal needs related to sexual violence.

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## About the Author

## Tony (Liuchen) Chen (University of Utah)

# 4. The More You Know: Knowledge About Gender as

## **Basis to Inclusive**

## Healthcare

## Practices

Natalia Garrido (University of Utah)

Faculty Mentor: Claudia Geist (Division of Gender Studies, University of Utah)

#### Introduction

Research has documented the many barriers to accessing healthcare and receiving quality, affirming care for gender and sexual minority individuals (Arthur et al., 2021; Blakey & Aveyard, 2017; Casey et al., 2019; Magnan et al., 2005; Weingartner et al., 2022). Discriminatory implicit or explicit actions or biases, whether they are institutional or interpersonal, are some of the barriers that work to oppress sexual and gender diverse (SGD) minorities from getting the health care they need (Everelles, 2011b; Farmer et al., 2006; Hana et al., 2021; Bauer et al.; Winter, 2016). Just by experiencing discrimination alone, there have been positive connections to poor quality of life and mental health (Jackson et al., 2016; Mays & Cochran, 2001).

To shed light on possible solutions to providing more inclusive healthcare environments, we focus on attitudes and practices among future healthcare professionals, the essential first impressions for SGD patients. In recent studies, pre-health students express mostly positive attitudes toward SGD individuals (Blakey & Aveyard, 2017; Bell & Bray, 2014; Kong et al., 2009; Magnan & Norris, 2008; Magnan et al., 2005; Arthur et al., 2021), but studies show throughout the health fields there seem to be a range of poor to no training or education measures on SGD health within school curriculums and occupations (Pratt-Chapman & Phillips, 2020; Obedin-Maliver et al., 2011; White et al., 2015; Eliason, 2017; Lena et al., 2002). Failure to understand and empathize with these complex differences and interconnections of SGD identities and health have left the SGD community drowning in life-threatening and chronic health conditions (Jackson et al, 2016; Operario et al., 2015; Rosario et al., 2014).

### Method

This study focuses on attitudes, views, and knowledge toward sexuality and gender via an online survey. The sample size contains 265 pre-health undergraduate students who have completed a specific amount of patient-centered hours and were enrolled in prerequisites courses for health programs.

## Results



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| We found mostly positive and supportive attitudes toward SGD |

individuals. 88% of students noted that hospitals should be a place to safely express and explore gender identities (FIGURE 1). The vast majority of participants also indicated that in their patient care experience, they attempted to use the correct pronoun all of the time and preferred name regardless of medical records. As well, 87% of students considered themselves an LGBTQ ally for their patients (FIGURE 2). However, not all pre-health students were comfortable talking to a non-binary patient and still tended to guess the patient's gender based on appearance.





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abstract topics of gender and sexuality, With most acknowledged the differences and binaries of gender and sex, but there was a constant undecided response (neither agree nor disagree) throughout the majority of the abstract questions. We found that 74% altogether disagreed with no difference between sex and gender and 7% altogether agreed while 19% neither agreed nor disagreed (FIGURE 3). For the question on sex being binary, 22% altogether agreed, 63% altogether disagreed and 15% neither agreed nor disagreed. The constant undecided response has further inquiries as to whether it is simply out of not wanting to choose, not knowing how to answer, or not understanding the question. Across the data of abstract topic questions, the more gender and sexual diverse a student identified, the more likely they noted they understood the complexities of sex and gender (FIGURE 5 & 6). Alongside understanding the complexities of sex and gender, it was found that mostly minority identities asked for pronouns.









It was also discovered that knowledge of SGD identities and their connection to inclusive behaviors contained a strong correlation. Though, we found stronger connections between inclusive behaviors with believing that sex is not binary and people being able to express more than one gender, compared to the knowledge that sex and gender are different.

## Conclusion

These views and attitudes toward SGD patients can help the healthcare community find ways where we can destignatize stereotypes and find the gaps in SGD health knowledge and training to create holistic healthcare. This data also further emphasizes that the attitudes of future healthcare providers are not the only foundations of exclusions in the healthcare system, but rather also a healthcare system and biased curriculum issue.

A reformation of the healthcare system should be the best option in making true holistic care. Steps must include

social views from healthcare examining providers, understanding the regulations in health care education that intentionally or unintentionally avoid inclusivity, pushing a new comprehensive educational narrative of health care, and sharing data about gender and sexuality with local health institutions and practices. But it is also still the responsibility of future healthcare providers to make room and push inclusive policies and practices. Future healthcare professionals will continuously be the first impressions of SGD patients and whether they should trust the healthcare providers and health institutions. Inclusive actions and welcoming environments of representation will make ิล difference for SGD individuals to feel safer in healthcare areas

In addition, this research, like many others, also further stresses the need to make room for SGD leadership in the reformation of the healthcare system. It is important to recognize that inclusion and diversity do not just mean only including sexual and gender minority individuals, but rather putting them in positions of leadership too. Giving SGD employees autonomy to work and change policies that promote inclusion and diversity removes any chances of changing policies and practices in a cis-gendered heteronormative way (Erevelles, 2011a). We must ask the leaders of SGD movements and SGD people in the community what they feel would make a better healthcare environment for them. Reformation must start within our local communities and it is everyone in the healthcare community's responsibility to push forward for an affirming, quality, and accessible healthcare for all.

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**DEPARTMENT III** 

# **School of Dentistry**

# 5. Optimizing Protocols for RNA Isolation From Fixed Tissue

Brenda Acosta (Utah Valley University)

Faculty Mentor: Melodie Weller (Dentistry, University of Utah)

Detection of pathogens in environmental specimens is challenging due to the rapid degradation of nucleic acids based on preservation techniques used. A study was designed to evaluate two protocols for specimen preparation for optimized preservation and integrity of cellular and viral RNA: 1) formalin-fixed paraffin embedded (FFPE) sample processing and 2) optimal cutting temperature (OCT) embedding compound for fixed and frozen tissue specimens. Soybeans in stage 1 of germination were fixed with 10% formalin for 24 to 48 hours prior to embedding in either FFPE or OCT. RNA was then isolated from 5um thick slices using a Qiagen RNeasy FFPE Kit. RNA quantity was measured with QubitTM RNA High Sensitivity (HS) Assay Kit and RNA integrity was assessed using QubitTM RNA IQ Assay Kit.

The objective of this study was to determine the optimal protocol for isolation of RNA from environmental samples. This study focused on the FFPE and OCT protocols. Each protocol had its own timeframe, challenges, and implications. FFPE required longer sample processing time, more reagents for step-wise tissue dehydration, and instrumentation to facilitate embedding specimens into hot paraffin wax. The OCT protocol required less time and reagents to process samples. FFPE and OCT protocols for RNA isolation were used to determine the optimal RNA concentrations and integrity of 24hr and 48hr fixed tissues.

In conclusion, the RNA concentrations for both FFPE and OCT were not significantly different (ns, p-value >0.05). Therefore, there was not a statistically significant difference between the embedding processes evaluated or for formalin fixation of 24 or 48 hours. Furthermore, no significant difference was observed between RIO score between FFPE and OCT methods or for fixation of 24 or 48 hours. Both FFPE and OCT samples rendered a similar RNA concentration and RIO score. The hours invested in each tissue embedding protocol were significantly different. FFPE requires a total of 96 hours to process the tissues, while the OCT protocol only requires 2 hours to process the tissues. For the future, the aim is to compare the FFPE and OCT protocols and observe if RNA is retained in situ.

About the Author

## Brenda Acosta (Utah Valley University) UTAH VALLEY UNIVERSITY

DEPARTMENT IV

# **College of Education**

6. Numbers and

**Nuclear Families:** 

**Gender and Work** 

in Indian Boarding

School

**Mathematics** 

## Curricula,

## 1879-1932

Luke Kim (Kenyon College); José Gutiérrez (Education, Culture and Society, University of Utah); Charles Sepulveda (Ethnic Studies, University of Utah); Kēhaulani Vaughn (Education, Culture and Society, University of Utah); and Cynthia Benally (Education, Culture and Society, University of Utah)

Mentor: José Gutiérrez (Education, Culture & Society, University of Utah)

### Abstract

In this paper, we address the thread of cisheteropatriarchal structures in the mathematics curriculum from the Indian boarding school system during the administration of Estelle Reel. Specifically, we focus our analysis on how gender is associated with housework in math word problems. We argue that these problems reflect how ideologies of gender oppression are a fundamental aspect and extension of settler colonial capitalism within the United States (Nakano Glenn, 2015). Theoretically, we draw upon prior Marxist feminist work that parse out the relationship between capitalism and patriarchy (Federici 1975; Vogel 1983). Further, we directly connect these theoretical approaches in the analysis of the mathematical content to broader U.S. federal policy and reformist efforts (Stremlau, 2005) during our period of study, 1879-1932.

### Introduction & Background

This paper presents an extension on ongoing research on math education in the Indian Boarding School System between 1879-1932 (Gutíerrez et. al, 2021) by focusing on how gender ideologies surrounding women were unmistakably central in the math word problems during the time. Elsewhere, we elaborate on how this content reflects federal policy of land dispossession (Smith et. al, 2022), which was also intimately connected to federal policies concerning indigenous women (Sepulveda, 2018; Stremlau, 2005). The aims of this paper are to 1) describe the particular ways in which gender appears in the mathematical curriculum of Indian boarding schools and 2) interpret the meaning of these problems within the context of larger settler colonial capitalist patriarchal systems to contribute to our understanding of how mathematics can act as a form of propaganda in reproducing these structures. We delineate our framework of analysis, methods, and significance in the following sections below.

### **Theoretical Framework**

TribalCRIT (Brayboy, Drawing upon 2005). Social Reproduction Theory (Vogel 1983, Bhattacharya and Fraser, 2017), and Evelyn Nakano Glenn's (2015) analyses of settler colonialism as the basis of race-gender formation in the United States, we suggest that the presence of cisheteropatriarchal ideologies in mathematics education (both during the time studied and now) are fundamentally grounded in the ongoing and ever-present structure of U.S. settler colonial capitalism and forced assimilation. In particular, one of the goals of U.S. federal assimilation policy at the time was to "fracture [...] extended indigenous families into male- dominant, nuclear modeled after middle-class, families. Anglo-American households" (Stremlau, 2005, p. 265), which required women's socialization into reproductive labor roles central to capitalist patriarchy (Federici 1975). By focusing on the thread of social reproduction in the boarding schools' mathematics curriculum then, this paper will demonstrate not only the indivisibility of this overarching structure, but also the necessity of critical

mathematics pedagogy approaches that pay due attention to the insidious practices of settler colonial capitalism that undergird education to this day.

## Methodology

As part of a larger ongoing historiography of mathematics education in Indian boarding schools in the late 19th and early 20th centuries (Gutíerrez et. al, 2021), our research team came across a wealth of archival evidence related to mathematics education in the Estelle Reel Papers from her time as the Superintendent of Indian Schools. The main data source utilized in this paper came from the "Arithmetic" folder, a collection of math curricula in the boarding school system, which most likely originated from Reel's summer training program for teachers around 1906-1907. Data consisted mainly of math word problems that were formulated for use in classrooms in different schools across the Indian education system, although there were some samples that followed more of a script that teachers would presumably use for interactive learning with their pupils (see tables).

Data analysis began with a process of open coding (Saldaña, 2012) and transcription of all materials to concretely observe general trends within the mathematics curriculum from the time. We are writing code books, identifying emerging categories representing distinct ways math problems reflect settler colonialism, and conducting statistical analyses based on the coding schema created from this process. We employed textual analysis methods (Carley, 1994) in the interpretation of problems that would fall into this purview to parse out the sociopolitical implications thereof.

## Analysis

With regards to the actual math problems themselves, gender, as it pertains to women, could generally be categorized

into two main groups. The first group, or what we term as *explicitly gendered*, encompassed problems that would mention unambiguously feminine-coded subjects (usually through names or through descriptors like "girls" and "women") following patriarchal norms in a clear-cut manner.

An example exercise that fell under this category is the following (see table 1 for more examples):

"If 1 girl washes 117 cups after supper, how many cups can 2 girls wash?"

The second, or what we named as *implicitly gendered*, included scenarios where some form of "feminine behavior" or a "feminine domain" as understood in a Eurocentric context was being exhibited, but without an immediately discernible subject and in some cases without a subject at all. These were generally more difficult to classify because of a lack of a definitive reference to women or girls, but could still be ascertained by drawing upon the cultural discourses of what was thought to be essential to "proper womanhood" at the time. A problem that might be grouped under this classification would be the following (see table 2 for more examples):

"<u>I washed 138 dish towels and ironed 50% of them.</u> How many dish towels did I iron?" (pg. 1)

Interestingly, however, was that across both groups a common through line in the representations and discourses of "womanhood" and "girlhood" was the primacy of reproductive labor (Federici, 1975; Vogel, 1983; Red Nation, 2020). The continual appearance of this thematic element is consistent with Trennert's (1982) study of indigenous girls' education in non-reservation boarding schools, where he notes that "[...] by the 1840s, the federal government had committed itself to educating Indian girls in the hope that women trained as good housewives would help their mates assimilate" (p. 272),

presumably into the dominant mode of production based on how "men's laziness" was a primary concern in these efforts. Thus, as a part of this larger attempt to enforce cultural assimilation among indigenous peoples into the growing capitalist society of the U.S., women's education was largely focused on housewifization (Mies, 1986), to the point that even mathematics curricula reflected this agenda.

Explicitly gendered problems were generally selfexplanatory, in the sense that these directly conveyed women and girls doing feminine tasks and thereby reinforced Eurocentric concepts of womanhood as tied to social reproduction. Even situations where the named feminine subjects did not do housework still had traces of reproductive labor in their content. For example, one exercise from a firstgrade class in Fort Shaw presented a scenario where a girl named Rose played with dolls and broke one. While this may seem like an innocuous inclusion, presenting the act of playing with dolls as feminine can be read as socialization meant to place care work as staunchly in the domain of "women's labor." In this way, Eurocentric capitalist patriarchal norms were highly salient in the math curriculum through constructing a Manichean gender system that placed women as the primary source of socially necessary labor in the maintenance and reproduction of the working class.

| Туре                   | Description   |
|------------------------|---|
| Explicitly<br>Gendered | Problems that directly mentioned girls and/or women in their content  |
| Implicitly<br>Gendered | Problems that either had an unclear<br>subject or no subject at all, but still<br>referenced discourses of femininity |

Figure 1: Explicitly Gendered vs. Implicitly Gendered

Like explicitly gendered problems, some of the implicitly gendered exercises were also straightforward in their content, albeit with the caveat that it wasn't immediately clear who was doing the domestic labor (see example above). However, problems without a subject at all posed the most analytically interesting situations, in how they carried the implication of the permanence of women's supposed role as reproductive laborers. Take, for instance, the following problem from a thirdgrade class at Haskell:

"There are 21 machines in the sewing room and each machine has 3 spools of white thread and 2 spools of black thread. How many spools of thread are there in all?"

In this case, while there is no subject doing "feminine" tasks, domestic labor still undergirds the mathematics practiced through asking students to count the number of spools of thread in the sewing room as an allusion to the production of clothing prescribed to women under capitalist patriarchy. And while this can be read just as a reference to the regime of domesticity in indigenous girls' education in boarding schools, the fact that "womanhood" was tacitly attached to the physical object of the sewing machine itself socialized girls into understanding the Eurocentric gender binary as а fundamentally real phenomenon.

Reproductive labor as it was configured in the exercises was also typified as either waged/paid labor or unpaid labor. Paid labor included situations where domestic duties and tasks were performed for some form of financial compensation, such as one problem from Fort Shaw where students were asked to find the total amount of money they would receive for doing the laundry of faculty members. As Gutíerrez et. al (2021) has already elucidated upon, the hyperfocus on vocational training in the boarding school system meant that any explicit math education indigenous students would receive would also be embedded in this larger scheme, with girls specifically having a curriculum oriented around domestic servitude for white settlers (Lomawaima, 1994). With this in mind, these paid labor situations in the mathematics curriculum can easily be read as a form of training to make indigenous girls fill subservient domestic positions for whites, with the understanding that their labor should be traded for settler currency.

Unpaid labor situations also involved the performance of reproductive labor, but without a named form of payment, as in multiple cases where girls would do housework presumably by virtue of their gender (see table 2). And though these unpaid contexts could be interpreted as another facet of girls' vocational training, they also arguably held an equally important role in socializing indigenous girls into the base unit of economic production in Eurocentric capitalism, that being the nuclear family. While at the time there were technically no federal policies explicitly focused on gender-based assimilation of indigenous peoples, family structures in the "civilizing mission" of the United States was a prominent and clear concern among white reformers at the time (Stremlau, 2005; Denetdale, 2017). As Estelle Reel (1901, quoted in Jacobs, 2005) herself put it, "The homes of the camp Indians are to be reached mostly through our schoolgirls, who are to be the future wives and mothers of the race, and on their advancement will depend largely the future condition of the Indian. All history has proven that as the mother is so is the home, and that a race will not rise above the home standard". However, a core tenet of the nuclear family structure is the prescription of domestic work as a devalued, mystified "labor of love" that women are effortlessly able to do (Federici, 1975). Taken together with

the eminent focus on families in reformer efforts, then, what appears is the idea that these unpaid contexts in the mathematics curriculum were a crucial part of conditioning indigenous girls into understanding themselves as naturally attuned to these tasks so they would later enter into the unpaid reproductive labor role in the nuclear family without much issue. At the same time, the undervaluing of these tasks also disrupted indigenous understandings of home maintenance as sacred and honorable, further contributing to the elimination of the native at the forefront of settler colonialism (Stewart-Haiwara, 2007; Tuck and Yang, 2012).

| Туре            | Description  |
|-----------------|--|
| Paid<br>Labor   | Problems where domestic (reproductive)<br>labor done was for payment of some<br>kind |
| Unpaid<br>Labor | Problems where domestic (reproductive)<br>labor was not compensated                  |

Figure 2: Paid Labor vs. Unpaid Labor

## Significance

This paper contributes to existing scholarship and activist efforts addressing gender inequality in mathematics education, as feminist analytics are more necessary than ever before. By demonstrating how capitalist cisheteropatriarchy was a fundamental part of the settler colonial project in Indian boarding schools, we urge educators and researchers in mathematics education and beyond to consider how gender in a U.S. context falls into the structure of settler colonial capitalism, and how even supposedly objective subjects like mathematics perpetuate this (Nakano Glenn, 2015; Arvin et. al, 2013; Federici, 1975). Furthermore, as opposed to trends in scholarly research that simply seek to describe the existing disparities in education and learning, we call for approaches that directly attempt to explain the material cause of these inequities and seek to critique the structures of power and exploitation that propagate oppression (Tuck and Yang, 2014; Mojab and Carpenter, 2017). In doing so, we hope that theoretical approaches in critical education studies, such as the one presented here, can be better linked to actual praxis in educational settings.

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## Appendices

## Appendix A – Tables

All problems cited in this paper are from the "Arithmetic" folder in the collection of the Estelle Reel Papers, Northwest Museum of Arts and Culture/Eastern Washington State Historical Society, Spokane, Washington.

Table 1: Explicitly gendered problems (original and<br/>transcribed problems)

Table 1: Explicitly gendered problems (original and transcribed problems)



## Table 2: Implicitly gendered problems (original and<br/>transcribed problems)

Table 2: Implicitly gendered problems (original and transcribed problems)



Table 3: Paid gendered labor (Original and transcribedproblems)

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Table 3: Paid gendered labor (Original and transcribed problems)



# Table 4: Unpaid gendered labor (Original and transcribed problems)

Table 4: Unpaid gendered labor (Original and transcribed problems) Sixteen cunces are one pound. " How many washings can you do with one pound of starch, when it takes one cunce forome washing? Sixteen cances are one pound. How many washings can you do with one pound of starch, when it takes one cunce for one washing? If Alice washed 6 stockings, how many pairs did she wash? If Alice washed 6 stockings, how many pairs did she was? I washed 138 dish towels and ironed 50% of them. How many dish towels did I #ron? I washed 138 dish towels and ironed 50% of them. How many dish towels did I iron?

## Appendix B – Code Book

Presents a preliminary coding schema, in which general themes emerged out of the math word problems from the Estelle Reel collection.

#### MATH CONTENT

ARITHMETIC: This grouping represents mathematics problems where students were asked to make use of the elementary operations of addition, subtraction, multiplication, and division
- SUMMING: Refers to instances where students were asked to sum/combine quantities with each other
  - ADDITION: Problems in this category would be focused on the application of addition through finding the sum of objects or money. While there may be some overlap with the multiplication category, this subcode tended to be differentiated because of its lack of the use of phrases like "groups"

## **Examples**:

"I had 11 oranges and my mother gave me 26 more. How many oranges have I now?" (pg. 3)

"If John can sew on 4 button and James can sew on 2, how many can both sew?" (pg. 11)

- SUBTRACTION: This represented instances where students would apply skills related to subtraction through finding differences. Questions tended to use phrases like:
  - "[...] much left"
  - "[...] many left"

## **Examples**:

"A man had 33 chickens and 6 were taken away. How many were left?" (pg. 7)

"There are three fish swimming in the river. George catches one fish. How many are there left?"

- GROUPING: This code represents problems where students were asked to think of quantities in terms of groupings through the skills of multiplication and division
  - MULTIPLICATION: This subcode encompassed problems where students would have to use skills related to multiplication through thinking about collections of objects as groups added together. A common idea was based around the idea of using a base rate to find a total, with multiplication questions phrased as:
    - "[abc] cents/dollars a [xyz]"
    - "[abc] objects a [xyz]"

Examples:

"I sell 15 cucumbers for 5 cents each. How much money do I receive?" (pg. 14)

"Find the cost of 28 pecks of potatoes at 85 cents a bushel." (pg. 1)

 DIVISION: This included instances in which division would be a central part of the problem, with students being asked to make as many equal groups as possible from a total number. Questions might be characterized by the phrase "is each".

Examples:

"If a farmer pays \$588.60 for 6 horses, how much does each horse cost?" (pg. 11)

"How many times can you take 2 eggs from 14 eggs?" (pg. 12)

- PROPORTIONS: This code represents problems where students would think of quantities as parts in relation to a whole
  - FRACTIONS: These problems would ask students to find a part or a number of parts of a whole group of objects or quantities, where the whole would be represented/abstracted as 1. Many of these problems would usually present a premise as "a person has/ does [fraction] of [quantity] – how many did they have". Questions may also ask students to combine different parts.

#### Examples:

"A watch cost \$28. and a chain 5/7 as much. How much did both cost?" (pg. 9) "One cup of meal needs 1/2 teaspon salt. Four cup of meal will need how many teaspoonfuls of salt?" (pg. 32)

> PERCENTS: Similar to the fractions subsubcode, students would be asked to find a certain amount of a whole, but in this case in the form of a percentage, where the whole would be represented/understood to be 100%.

Examples:

"I washed 138 dish towels and ironed 50% of them. How many dish towels did I iron?" (pg. 1)

"Mr. R.J. Brown bought 24 head of cattle for \$1200. and sold them. He estimated his net gain at \$10. a head, 20% of which was due to extra care feeding. What would have been the selling price per head if he had not taken the extra care?" (pg. 23)

- MISCELLANEOUS: This subcode encompasses mathematical skills that would not fall into the defined arithmetic skills above but still featured as a common theme in the archival evidence. Other mathematical skills not covered in this code book would fall into this category, just without a subcode
  - CONVERSIONS: This sub-subcode would encompass problems where students would switch quantities in one type of unit to another. Units were always in the imperial system used in the United States, although some of the units may be unfamiliar to viewers today.
    - CONVERSIONS [simple]: These questions typically were more focused on memorizing the actual value of units, and would usually only involve converting one of a unit to another

Examples:

"How many inches in a foot?" (pg. 17) "How many cents make one dime? (pg. 35)

 CONVERSIONS [complex]: These questions also assumed a base knowledge of imperial units, but were more complex in the sense that they would typically require the use of arithmetic skills (such as multiplication or division) as opposed to a solely memory based task

Examples:

"How many qt. in 5 1/4 gal.?" (pg. 35) "How many eggs in 3 1/2 doz.?" (pg. 34)

#### SOCIOPOLITICAL CONTENT

LAND: This code refers to content where students were asked to solve problems in scenarios in which land was a central focus. In many cases, the unit of measurement prioritized was the acre, but there were other instances in which measurements of length (typically either in miles, yards, or feet) were also used. (Measurement itself also constitutes a political act)

> • LAND [Property]: Stated or alluded to a belief that physical land could belong to a person or a group of people. These problems would either explicitly have a character/person possess some amount of land or would imply it through other means (usually through transactions). Common phrases would include:

• "...had/has/owns... acres..."

- "...bought/sold... acres..."
- "allotments/allotted"

Examples:

"I have a field of alfalfa of 10 acres." (pg. 27)

"A man bought a quarter section of land (160 acres) at \$10. per acre." (pg. 12)

 LAND [Modification]: Instances where land would be changed or adjusted in some way, typically for the purposes of agricultural or animal husbandry purposes. Common structural themes would have some type of action done to the land, such as "irrigates", "tilled", or "planted"

Examples:

"About how much would it cost to irrigate one acre if we had to pay 12 cents per inch? (one acre takes 8 inches." (pg. 13) "The Haskell farm contains 1000 acres. 30 acres were planted in potatoes. What part of the farm was planted in potatoes?" (pg. 1)

LABOR: This code refers to problems where students would be asked to do some form of work to provide the means for subsistence/everyday living. Different mathematical skills were integrated into these problems, but most of the labor was manual/blue-collar work. Gender was highly salient in the types of labor that would be prescribed in these problems following Eurocentric patriarchal norms.

- LABOR [men's work]: Encompasses work related problems that would focus on labor that is gendered as masculine, usually things regarding tasks that would be done outside of the home.
  - LABOR [men's work worker owned]: Problems would usually have students imagine themselves in scenarios where the products of one's labor would be owned by the worker themselves, usually in agricultural or animal husbandry work. These products could then be traded for currency in these types of problems.

Examples:

"If you plant 3 rows of onions and raise 24 onions in each row, how many dozens of onions will you raise?" (pg. 19) "If you have a field of 80 acres and it costs \$1.12 1/2 an acre to plow it, 25 cents an acre to pulverize and harrow it, 35 cents an acre to plant it, the seed corn costs \$1.00 per bu. And it takes 1 bu. to plant 4 acres, find the cost of seed and planting" (pg. 20)

> LABOR [men's work – waged]: This subsubcode would ask students to imagine themselves trading their actual labor for some amount of money, usually set to an hourly/daily wage of some kind.

Examples:

"If a man can pick 210 lbs. cotton in 1 day, how many lbs. can he pack in 3 days and how much will he receive for picking at

## \$1.25 per cwt?" (pg. 24)

"If we pay each man \$1.50 per day, how much do we pay 5 men for 1 day? For 7 days?" (pg. 10)

- LABOR [women's work]: Problems where the labor done would be related to domestic labor and household management, usually involving cleaning and cooking in some capacity. These tasks would usually be prescribed a "feminine" quality in line with Eurocentric ideals of gender.
  - LABOR [women's work waged]: contexts where domestic labor would be done in service of someone else, with the worker earning a certain amount of currency in exchange

Examples:

"If I do the employees' washing and Mrs. Lovewell pays me 20, Miss Whisnant pays me 18, Miss Benn pays me 21, Miss Benton pays me 27 and Miss Davies pays me 15, how much do I earn?" (pg. 4)

"Mary made 8 cents making a bead necklace. How much did she earn making 3 bead necklaces?" (pg. 5)

o LABOR [women's work – unwaged]: problems where the tasks of cleaning and cooking and other household duties would not be given a monetary compensation

Examples:

"In the laundry we put 65 sheets in the washers. There are three washers. How many sheets can we wash in five days? We fill the washers three times every afternoon. Each washer holds 65 sheets at a time. How many sheets is that altogether?" (pg. 2)

"Mary made 8 cents making a bead necklace. How much did she earn making 3 bead necklaces?" (pg. 5)

> LABOR [women's work – unwaged]: problems where the tasks of cleaning and cooking and other household duties would not be given a monetary compensation

Examples:

"In the laundry we put 65 sheets in the washers. There are three washers. How many sheets can we wash in five days? We fill the washers three times every afternoon. Each washer holds 65 sheets at a time. How many sheets is that altogether?" (pg. 2)

CAPITAL/COMMODITIES: This code includes problems that were centered on currency or goods that would be exchanged for each other, usually either finding change or the dollar amount of certain units of currency, or selling and buying objects for amounts of money. Importantly, all of these were focused on U.S. currency. page12image12072640

- CAPITAL/COMMODITIES [currency]: problems where currency issued by the U.S. government featured as a primary part – key words and symbols include:
  - "\$/dollars"
  - "cents"
  - "nickels/dimes/pennies/etc." (actual units of currency)

Examples:

"A boy had 10 cents and his mother gave him 9 cents more. How many had he then?" (pg. 4)

"How many dimes in a dollar?" (pg. 36)

"How many cents are 2 dollars, 2 nickels, and 1 penny?" (pg. 38)

- CAPITAL/COMMODITIES [interest]: Problems where money buy itself would increase in value by virtue of it being money. These usually included an interest rate in the form of a percent, where the value of a sum of money would increase over time. Key phrases/terms would include:
  - "...principal/interest/rate of interest..." o "...loaned/borrowed..."
    "...investment..."

Examples:

"A man had a certain sum of money he wanted to earn \$75.40 at 4 12% in 3 yr. 4 mo. What was the sum?" (pg. 5)

"The Farmer's National Bank of Carlisle pays 3% interest, savings account, compounded semi-annually. If a boy at the age of 18 should deposit \$96, how much would he be entitled to when he became 21 years old?" (pg. 23)

MISCELLANEOUS: This code includes any problem content that wouldn't fit into the main three categories presented above, but still may carry some significance with regards to the sociopolitical and economic context of the colonial machinations of the US at the time.

• MISCELLANEOUS [animals]: Problems of this type would reference non-human animals in some

capacity, such as pigs, sheep, horses, etc.

 MISCELLANEOUS [animals – property]: Instances in which nonhuman animals were presented as something that could be owned by a human entity, either explicitly or implicitly (through buying and selling)

Examples:

"A man had 13 sheep in a field and 3 of them died. How many sheed has he now?" (pg. 4)

"Mr. Brown bought 18 horses at \$175. each. He sold them at \$200. each. How much did he gain?" (pg. 21)

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**Arithmetic: Land** 

**Dispossession in** 

**Indian Boarding** 

School

**Mathematics** 

# Curricula,

## 1879-1932

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### Abstract

This paper uses Holm's (2005) framework of the three phases of colonialism to analyze mathematical word problems in the Estelle Reel Papers. Our main findings reveal that the mathematics instruction in Indian boarding schools reflected the tactics used across the three phases. Analysis of the social and political content embedded in these word problems also indicates a connection to the idea that teaching private property ownership would force Indigenous people to abandon their cultural heritage, develop a sense of individualism, and empower a sense of freedom in the owner. This idea of private property ownership is antithetical to the views of many Indigenous peoples, who draw a deep connections to life through land and the environment (Sepulveda, 2018).

### **Objective/Purpose**

This study is part of a larger project examining federal policies of cultural assimilation and mathematical content taught in Indian boarding schools during the early 1900s (Gutiérrez et al., 2021). This paper focuses on mathematics problems referring to land and how these characterizations are antithetical to many Indigenous views. The aim of this paper is to show how assimilation practices embedded in the mathematics problems in the Estelle Reel Papers were used to reconstruct the way Indigenous people viewed land.

#### **Perspective/Theoretical Framework**

This project works to fill the gaps in Indigenous histories pertaining to mathematics in Indian boarding schools that have not been explored. Utilizing a critical analysis, we highlight how the math curriculum was used to assimilate Indigenous students by attempting to strip their sacred relationships to and understandings of land. Theoretically, this paper draws on the work of Indigenous and anti-colonial scholars—Holm (2005), Ornelas (2007), Sepulveda (2018), and Tuck & Yang (2012) to interpret math word problems as instances of internalized colonization.

#### **Phases of Colonialism**

Holm's three phases of colonialism are a reflection of the Indian-White relationship (Holm, 2005). Holm breaks down these phases into three steps, each building off the previous.

Phase 1 – Disruption and Boundary Making: The first of this phase is the disruption and displacement of Indigenous peoples. Holm describes this as the start of a conflict between the Indigenous people and colonizers. This process typically leads to an agreement that is meant to set territorial boundaries and maintain order (Holm, 2005). However, since the goal of the colonizer is to acquire land, these agreements with Indigenous communities (treaties) are often broken, resulting in more violence and disorder (Holm, 2005).

Phase 2 – Forced Assimilation: The second of these phases involves the attempts to integrate Indigenous people into a colonial socioeconomic structure (Holm, 2005). Here, assimilation through forced labor or teaching Indigenous people to speak the colonizer's language is used (Holm, 2005). Phase 3 – Internalized Colonialism: Finally, following Albert Memmi, the final phase of colonialism is internalization (Holm, 2005).

Indian boarding schools were created in the late 1800s, around the same time the Dawes General Allotment Act was passed in 1887. The Dawes Act dispossessed Native people of over 90 million acres of land and allowed the government to assign land allotments to Native families. This idea of land dispossession followed into the education system in Indian boarding schools, which is visible in the mathematics problems. In this way, the U.S. integrated assimilation into the education system by teaching students about private ownership and inculcating them with those values.

#### **Anti-Colonial Perspectives on Land**

Within Native perspectives, the importance of land is seen as a human rights issue (Ornelas, 2007), where humans have a sacred responsibility to care for their Mother Earth (Sepulveda, 2018). This view of land embeds a spiritual understanding that interconnects culture, spirituality, and religious difference (Ornelas, 2007). This perception of land is antithetical to Euro-Western Christian worldviews, which put a strain on the policies made to support and protect lands (Ornelas, 2007). Additionally, Sepulveda (2018) links settler conceptions of land as akin to their beliefs about Indigenous people: uncivilized and ready for domestication (Sepulveda, 2018). Settlers transform land into property through the process of settler colonialism and restrict relationships to land strictly as property owners (Tuck & Yang, 2012).

These views on land play an integral part in analyzing the mathematics problems in the Estelle Reel Papers as a form of colonial curriculum. In Indian boarding schools, Native relationships to land were transformed into the settlers' way of thinking, and the idea of land ownership was prevalent in the mathematics education taught in Indian boarding schools.

#### **Methods and Data Sources**

As a part of a larger, ongoing project, this paper focuses on the land aspects related to the math word problems used in the colonial curriculum in Indian boarding schools. The original project (Gutiérrez et. al, 2021) set out to investigate assimilation practices in mathematics curricula in Indian Boarding Schools. During the data collection phase, the research team discovered mathematics word problems in the Estelle Reel Papers, which is a well-known collection among historians of the Indian boarding school system. These primary materials consist of arithmetic problems that were presumably created by teachers and pupils and submitted to Estelle Reel, who was the superintendent of Indian schools between 1898-1910. These word problems were meant to showcase the mathematics instruction that young Native students were receiving. The math problems reflected in this paper come from a subset of the data: problems aimed at teaching first through eighth-grade students. To launch our analysis, we transcribed the word problems to develop code books and a database. Specifically, word problems pulled from the database involved terms such as allotments, land, cultivation, and fencing. Upon further evaluation, various themes emerged (Saldaña, 2016) from these word problems that were consistent with the idea of assimilation.

A preliminary draft of emerging codes can be found in Appendix A. The code book presents common themes emerging from the mathematics problems and draw out ideas for other research foci. The transcriptions we have made will be used to develop a statistical analysis of the mathematics problems in the future. For now, this paper presents a qualitative analysis of some of the major themes that have emerged thus far. We qualitatively analyze word problems focused on relations to land and how they were used to colonize Indigenous students' relationship to land.

#### Results

By focusing on the math problems that are related to land in the collection, three themes arose. These themes are consistent with Holm's first two phases of colonialism that are ultimately planned to achieve the third and final phase. The first of the themes that were found was the connection to the Dawes Act of 1887. These mathematics problems explicitly involved scenarios where the students were to be 'given' a piece of land. This can be seen in the following problems (Note: all problems cited are from the "Arithmetic" folder in the collection of the Estelle Reel Papers, Northwest Museum of Arts and Culture/ Eastern Washington State Historical Society, Spokane, Washington):

"When you get your allotment [sic] of 160 acres and want to fence it, how much will it cost you if it lines in a square, with a four wire fence, putting the posts 1 rod apart; the posts being work 9¢ apiece and the wire 4¢ a rod."

"If you are allotted 160 acres of land and rent half of it for 10¢ per acre, how much money will you receive?"

During this time, land was emphasized as a commodity utilized for profit in white American views instead of land as ancestor. For example, in the 1916 Annual Report, Indigenous people who received allotments were urged to use them to make a home and "work the lands" (Sells, 1916, p. 30).

These ideals give way to the second prominent theme: modifying land for its 'betterment'. These word problems convey the idea that land itself was not sufficient and needed to be utilized for farming or profit-making. "The farm detail at the White Earth boarding school cleared a tract of land for cultivation of the following dimensions: Length 251.6 ft., width 185 ft. What part or parts of an acre does the cleared tract add to the school farm?"

"I have 45 acres of land. Clearing off sage brush costs \$2 per acre; leveling cost \$1. per acre; plowing \$2 per acre; water 25¢ per acre; seed 1-1/2 bu. per acre at 85¢; seeding per acre \$1 and harvesting \$2 per acre. What did it cost me to plant and harvest my wheat? The yield was 15 bu. per acre, at 85¢ per acre. What did I sell it for? What did I gain?"

These problems clearly demonstrate the idea of land modification for cultivation. This aligns with the notion that white settlers' interests were to transform the lands to use for their needs (Sepulveda, 2018) since their identities come from making 'productive' use of the land (Tuck & Yang, 2012). However, in the first problem, it is also important to note that the question asks how many acres will "add" to the school farm, creating the concept of land ownership. Rather than stating the question as "how many acres will the cleared tract create," the word problem creates the notion that the cleared land has to belong to an owner. The second problem also incorporates this idea. Rather than stating a piece of land is "45 acres," the problem explicitly mentions that it belongs to me (the reader), where both problems further pursue Tuck & Yang's argument that settler colonialism transforms land into property and that human relations to land are as its owner (Tuck & Yang, 2012).

This leads to the last theme: land as private property. These mathematics problems incorporated the use of land as one's property ownership by 1) creating an enclosure around the section of land to signify private property and prevent accessibility, and 2) through land purchasing. This can be seen in the following: "How many rods of wire will it take to make a 3-wire fence around a field a mile square?"

"If I receive \$35. a month and spend \$20. a month, how long will it take to buy 20 acres at \$40. an acre?"

The top problem involves the use of private property by creating an enclosure to show ownership. The second word problem involves labeling land as a currency to be purchased. Here, the idea of having worth emerges. Notions of monetary worth also appear in other word problems, which we examine more closely in another study focused on gendered wages and labor (Kim et al., 2022). Overall, the notion of worth highlights that Western ways of thinking involved the constant need to value an object. This need to value land was familiar to white settlers since they viewed land as a source of capital and were convinced "wild land" was made for their benefit (Tuck & Yang, 2012).

#### **Scholarly Significance**

These problems highlight the math education that attempted to strip the sacred relationship that Indigenous students had to land. However, it is important to note that this idea was not only taught in the word problems. Severing sacred relations to land was accomplished in other areas of the school day, went beyond the math curriculum, and continued after EstelleReel's time as superintendent.

For this paper, we focus on the vocational division where students, seventh through tenth grade, began their studies in agriculture and homemaking (Sells, 1916). In the 1916 Annual Report (Sells, 1916), courses of study were developed to teach students in Indian boarding schools called the vocational division (see Appendix B for images of the Outlined Courses of Study). Here, students had courses in "Industrial Geography and Agricultural Botany" (seventh grade), "Vocational Arithmetic and Farm and Household Accounts," and "Soils and Soil Fertility" (eighth grade). These types of courses continued into their final year, where classes like "Rural Economics, and Insects and Insecticides" and "Field Crops and Plant Diseases" were implemented as a course of study. In addition, students were to have 4 hours of "Industrial Work" where they would apply their lessons from these classes. These lessons were introduced and experimented with in all U.S. Indian boarding schools in 1916 before it was to be permanently published (Sells, 1916). Given this, the course of study further shows that the true lesson in Indian boarding schools was to teach students about vocational work as a way to strip their sacred relations to land and redirect their loyalties to the colonial system (Holm, 2005).

The significance of this work is to fill an incomplete history of Indigenous peoples and Indian boarding schools. At the same time, the hope is that this paper highlights implications for teacher education by challenging educators to reexamine their positions in the classroom, the areas we live in, and the time we live in. From a distance, these math word problems might not appear to involve cultural assimilation, but by deconstructing the math word problems and analyzing the time, space, and people they were written for, it is clear they did. This work further challenges the idea that math is objective by showing that mathematics education can be a weapon for assimilation (Bishop, 1990; D'Ambrosio, 1985).

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#### Appendix A

Preliminary Draft of Code Book

ARITHMETIC: Refers to mathematics problems involving fundamental operations on numbers to get an answer. Generally, these problems involve statements or expressions consisting of addition, subtraction, division, or multiplication.

- "How many..."
- "How much..."
- ARITHMETIC [summation]: This subcode is a counting problem that uses simple addition/ subtraction problem with one solution.
  - "I had three dozen pencils and gave the class 13. How many were left?"
  - "There were 18 marbles in the box. The boy took 2 marbles. How many marbles were left?"
- ARITHMETIC [money]: Simple summing problem that involves American currency(explicitly mentions it).

o "If you have three dimes and a half dime, how many cents have you?" o "How many oranges at 3¢ each can you buy with 10¢?"

- ARITHMETIC [memorization]: This subcode requires an answer that is based on a definition, rule, or table that is expected to be memorized.o "How many cents in one dime?"
  - "Define the terms of percentage."
  - "What are the dimensions of a roll of paper?" o "Whatisinterest?"
- ARITHMETIC [multiplication]: This subcode requires problems that often involve a given number

that is to be multiplied or divided by another number to achieve a value.

- "If there are 12000 pieces washed in one week at the laundry, how many pieces will be washed in 16 weeks?"
- "If Haskell received 780 boxes of shoe blacking, worth 40 cents a dozen boxes, how much did it all cost?"
- "Reduce 144/216 to lowest terms."
- "5/8 of 9/20 = ?"
- ARITHMETIC [percentage]: This subcode uses ratios or fractions, whose denominator is always 100, in the form of percentages
  - "Twenty-five words were given in the spelling lesson. Charlie was marked88%. How many words did he misspell?"
  - "If you have \$500, and spend 10% of your money usefully, how muchhave you left?"
  - "A farmer sold 12 cows. This was 20% of all he had. How many had he atfirst?"BUSINESS: This code refers to problems that are involved in trades, transactions, or buying/selling using U.S. monetary currency. They often use terms such as: net worth,

gain, profit, and value.

- BUSINESS [value]: Refers to finding the worth or value of an item.
  - "If ice is worth 45¢ per 100 lbs., find the value of the ice and net worth."
- BUSINESS [profit]: Involves the action of selling or buying items with an explicit focus on financial gains—i.e., profits.
  - "If a man pays \$25 for a cow and sells her for \$40, how much does he gain?"
  - "I bought 12-1/2 yards of ribbon at 25¢ a yard, and sold it at a gain for \$1.12-1/2, what was the selling price?"
- BUSINESS [interest]: Problems are based on investing money and/or calculating values based on interest rates.
  - "Find the simple interest on \$500 for 3 yr. 6 mo. at 7%?"
  - "What is the interest of \$250.00 at 6% from July 3, 1902 until Feb. 5, 1906?

AGRICULTURE: This code refers to word problems that involve animals, farming, material, and crops.

- AGRICULTURE [commerce]: These types of problems involve agriculture that is used for buying and selling.
  - "I raised 18 bushels of turnips but I

sold one half of them. How many bushels did I sell? How many had I left?"

- "A farmer sold 37-1/2% of his crop of 816 bushels of wheat at \$1.56 per bushel, and the rest at \$1.60 per bushel. How much did he get for his entire crop of wheat?"
- AGRICULTURE [animal]: This subcode refers to problems that involve animals and selling/buying them.
  - "I paid \$64 for 8 pigs. How much did I pay for each pig?"
  - "If 6 sheep cost \$30, how much will 20 sheep cost?"
  - "At 25¢ each, how many chickens will be required to pay for 400 lbs. flour at \$3 per cwt.?"
- AGRICULTURE [material]: Refers to math problems that involve the use of materials that are found in nature.
  - "How much will a pile of cord wood cost 40 ft. long, 16 ft. wide, 6 ft. high at \$4.50 per cord?"
  - "If it requires \$6.00 worth of wood to make one carriage, how many carriages can be made for \$20.00 worth of wood?"

LAND: This code refers to math problems that involve

scenarios that are intended to be familiar to the student in terms of land.

- LAND [private property]: These are mathematics problems that involve land ownership.
  - "When you get your alotment [sic] of 160 acres and want to fence it, how much will it cost you if it lines in a square, with a four wire fence, putting the posts 1 rod apart; the posts being work 9¢ apiece and the wire 4¢ a rod."
  - "If you are allowed 160 acres of land and rent half of it for 10 cents per acre, how much money will you receive?"
  - "A man has 24 acres of ground; he planted 6 acres in corn and 6 acres in wheat? How many acres of ground did he have left?"
- LAND [modification]: Situations that ask the student to "modify" land with materials.
  - "A field is 60 rods long and 40 rods wide; what will it cost to fence it at 15¢ a rod?"
  - "My field is 924 feet long and 338 feet wide. At \$4.25 a hundred pounds, what will the wire cost to enclose it with a 3 wire fence?"
- LAND [rent]: Builds off of the idea of land as property, but goes further to sell/rent off pieces of

the given land to make a profit.

- "If you are allotted 160 acres of land and rent half of it for 10 cents per acre, how much money will you receive?"
- "A house that cost \$2500.00 was rented for \$325.00. If \$150.00 was made for repairs, what rate of interest did the owner receive on his money?"LABOR: This code refers to math problems that involve manual labor to finish a domestic project. These problems often use mixed mathematics to carry out the project involving materials and have gendered associations.
- LABOR [men]: This code refers to men who perform manual labor through farming, carpentry work or physical labor
  - "How many feet of wire will Charlie need to fence a garden 10 rods long by 8 rods wide, if he puts three strings of wire around it?"
  - "Pedro receives one dollar per day for pruning trees on the Gould Ranch, how much will he earn in three weeks?"
  - "If a man can plant 2/5 of his farm in 3 days, how long will it take him to plant all of it?"

- LABOR [wage]: Refers to problems that involve wages or compensation men receive for a type of work.
  - "The labor of a man and team is worth \$3/50 per day. How much will it cost a farmer to have an irrigation ditch dug, if it takes 8 days to dig it?"
  - "Willie earned 25 cents Saturday afternoon chopping wood. He bought 2 handkerchiefs for 5 cents each. How much money has he left?"
  - "James carries coal for 3 employers and receives 10 cents a week from each. He also earns 5 cents a week by selling "Saturday Evening Posts". How much does he earn in all in one week?"
  - "Vincente earned \$7.58 a week working in the best fields. If his board cost him \$8.25 a month, how much money had he at the end of two months."
- LABOR [women]: This code involves women who are creating domestic products or household work as labor.

• LABOR [cultural referencing]: This

subcode refers to math problems that make references to Indigenous arts/craftwork to sell and profit from.

- "If Mary makes two beaded belts in ten days, and sells them for \$2.50 each. How much did she earn per day?"
- "Mary sold an Indian basket for \$2.40 which was 10% more than it cost her. What was the cost?"
- LABOR [material]: This subcode involves women who are making products from various materials.
  - "If a woman makes a basket worth \$2.50. How many lbs. of coffee at 25¢ per lb. can she get for it at the store?"
  - "It takes 3 yds. of gingham to make a dress for Emma and 2 times as much to make one for Jennie. How many yards will it takes to make a dress for Jennie?"
  - "There are 19 girls in Company D and it takes 3 yds. of gingham to make school aprons for each. How many yards will it take to make aprons for

#### all?"

MISCELLANEOUS: This code refers to math problems that do not have a math answer. They are open-ended rather than require calculations.

- "Mary how many pints of water can I put in this pan? (Mary does not know). You may measure. What did you do? Give me another name for three pints?"
- "We are to paper, carpet, and furnish a three-room cottage, the dimensions of which are as follows:... What is the first thing to be done?...What are the dimensions of a roll of paper? How many sq. ft. in a roll, then? Will the merchant give you a fraction of a roll? ...How shall we have the strips of this carpet run?.. Shall we carpet the other two rooms? What may be done with that?" [Note: This problem is part of a larger one that was too long to include here. The relevant parts of the problem are included instead.]

#### Appendix **B**

Courses of Study found in the 1916 Annual Report of the Commissioner of Indian Affairs to the Secretary of the Interior, for fiscal year ended June 30, 1916

Image 1b. Image of the Outline of Course of study for first year students in the vocational division

#### 78 Annie Isabel Fukushima (Editor in Chief)



Image 2b. Image of the Outline of Course of study for first year (continued) and second year students in the vocational division

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Image 3b. Image of the Outline of Course of study for second

year (continued), third year, and fourth year students in the vocational division



Image 4b. Image of the Outline of Course of study for fourth year (continued) students in the vocational division





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**DEPARTMENT V** 

# College of Engineering

## 8. Reflection on

## Undergraduate

#### Research

### **Experience** -

## **Andrew Bergenthal**

Andrew Bergenthal (University of Utah)

Faculty Mentor: Sameer Patil (School of Computing, University of Utah)

My SPUR [Summer Program for Undergraduate Research] experience was primarily driven towards a cybersecurity related topic. This is going to help impact my future education in goals mainly through the experience itself and its contribution to my resume. Research was never something I had initially planned on doing, however, I found this to be a valuable experience through the research perspective. In the future I plan to eventually work in Augmented Reality related fields and this type of research will presumably occur on a daily basis so it has been a great help.

About the Author

Andrew Bergenthal (University of Utah) UNIVERSITY OF UTAH

## 9. Optimization of

## **Brain Slice Health**

## for Ultrasonic

## Stimulation

Gabrielle Bingener (University of Utah); Jan Kubanek (Bioengineering, University of Utah); Carena Cornelssen (University of Utah); and Peter J. West (Pharmacology, University of Utah)

Faculty Mentor: Jan Kubanek (Bioengineering, University of Utah)

There is a critical need to develop noninvasive treatments for drug-resistant neurological disorders. Focused ultrasound is a noninvasive modern technology that can provide mm precision

and target deep brain structures. Yet, it is unknown the effects of ultrasound on different cell types. Our goal is to develop a method which we can determine the calcium response in neurons to ultrasonic stimulation. When optimizing a method to image calcium response, it is crucial to ensure brain slice health as cell death can occur during preparation of brain slices and in the imaging environment. We established using Thy1-GCaMP6s animals because of its increased fluorescence in CA1 hippocampal cells (Dana et al., 2014) and greater detection of active neurons (Chen et al., 2013). Using a Zeiss confocal microscope imaging at 12 Hz of coronal sections of Thy1-GCaMP6s (n=2), we determined that the NMDG protective recovery method for slice preparation and our solution process was sufficient. This success is marked by imaging predominantly healthy cells with full soma. During imaging coronal Thy1GCaMP6s (n=2) with an Olympus BX51 illumination turret, 10x water immersion .3NA objective, and GFP filter set, we determined the recording temperature of 30-32 °C and 7.35 pH was sufficient for healthy brain slices as observed by non-bloated cells in the imaging environment. Additionally, we recorded continual brain tissue viability for a minimum of six hours. Optimization of slice health and the imaging environment for the tissue will allow us to create a novel setup for ultrasonic stimulation of brain slices.

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## 10. Production of High-Purity Calcite and Rare Earth Elements from Phosphogypsum Rachel D'Agostini (University of Utah)

Faculty Mentor: Xuming Wang (Materials Science and Engineering, University of Utah)

Phosphogypsum (PG) is a solid waste from phosphoric acid production, and is primarily composed of  $CaSO_4.2H_2O$ . It is estimated that about 200 Mt/a of PG is generated globally in the phosphate industry (Parreira et al. 2003). Due to impurities (such as phosphate, fluorides, sulfates, trace metals and radioactive elements) only 15% of the PG is recycled as building material and soil stabilization amendments (Tayibi et al. 2009). Most of the PG is stored in large stacks across the world. The large number of PG stacks is not only occupying land space, but is also an environmental problem (Rutherford et al. 1994, 1996). On the other hand, the PG could be a material resource. For example, research indicates that the content of REE (Rare Earth Elements) in PG is about 112-300 ppm (Zhang 2014) which is a significant REE resource.

Current study for recovery of REE from PG using NH4Cl dissolution is in progress. In addition, preparation of high purity of CaCO<sub>3</sub> through CO<sub>2</sub> mineralization is included in our research (Figure 1). The processing parameters including dissolution time, temperature, and liquid/solid ratios are being explored to determine the optimum conditions. Fouriertransform infrared spectroscopy (FTIR) is being used to examine the products, and a Ca++ ion selective electrode is being used to observe dissolution and carbonate formation processes. The preliminary results show the feasibility of using the PG dissolution - CO2 mineralization process to recover REE and produce CaCO3 from PG. The advantage of this processing is to use the solid waste PG and greenhouse gas CO<sub>2</sub> as feed materials to produce valuable products, REE and CaCO<sub>3</sub> Future research will include extraction of REE from solution







Figure 1.  $CaCO_3$  product from phosphogypsum by using PG dissolution –  $CO_2$  mineralization processing.

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About the Author

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## 11. **Joint**

**Classification-Regr** 

#### ession Algorithm

#### for Myoelectric

#### Control

William Caden Hamrick (Mercer University); Jacob George (Electrical Engineering, University of Utah); and Connor Olsen (University of Utah)

Faculty Mentor: Jacob George (Electrical Engineering, University of Utah)

The purpose of this research is to develop a joint classification-regression scheme to provide consistent, accurate and proportional myoelectric control of smart-home devices. Myoelectric control

uses electrical recordings from the surface of the skin to provide intuitive and natural control of assistive devices. Most myoelectric control algorithms either classify discrete actions (press one button at a time, like a keyboard) or provide proportional (regression) control of a a few actions (slide in one direction, like a mouse). Here, we introduce a new algorithm that uses a classifier to select one from a pool of regression models each trained on a singular action. We employ a Convolutional Neural Network (CNN) to classify myoelectric input as one of three gestures: 1) rotating a dial, 2) swiping downward, 3) doing nothing (i.e., hand at rest). In addition, we train two Kalman filters (KF) for regressing the degree an individual rotates or swipes. Preliminary results show 95% classification accuracy and a root mean squared error of .175 with regression. This joint classification-regression scheme provides a unique solution to a novel problem of selecting among discrete gestures that each have proportional classification-regression The joint outputs. algorithm may provide better overall performance than state-of- the-art classification and regression algorithms for myoelectric control. The results presented here also provide one of the first early demonstrations of myoelectric control for smarthome devices

About the Authors

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## 12. Reflection on

### Undergraduate

#### Research

## **Experience** -Josh

## Hillyard

Josh Hillyard (University of Utah)

Faculty Mentor: Allison Payne (Bioengineering, University of Utah)

My undergraduate research experience has contributed immensely to my education as a future engineer. The impact this has given me is a goal to eventually go back to school for a master's [degree]. This would not be the case had I not been given an opportunity to work in academia and be involved with this scientific community here at the U.

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About the Author

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13. Utilizing

**Non-Invasive** 

Focused

Ultrasound to

Improve

Immunotherapy

for Triple Negative

#### **Breast Cancer**

Josh Hillyard (University of Utah); Allison Payne (Bioengineering, University of Utah); and Sara Johnson (University of Utah) Faculty Mentor: Allison Payne (Biomedical Engineering, University of Utah) and Sara Johnson (University of Utah)

Checkpoint inhibitor immunotherapy is an emerging treatment option which has shown to be incredibly successful across multiple cancer types and stages. It functionally serves to block certain cell membrane proteins on immune cells and facilitate their efforts towards killing off tumor chemotherapy cells. In contrast to and radiotherapy, immunotherapy targets specific cell populations, minimizing systemic toxicity. In the clinical setting of breast cancer however, response rates for patients are modest at best. This can be inadequate infiltration explained by and communication between critical immune cells such as T lymphocytes, natural killer cells, and dendritic cells. Hormone receptive/HER2 negative breast tumors also have vasculature and stromal barriers contributing to the overall poor response. Therefore, significant interest exists in reshaping the tumor microenvironment while administering an immunotherapeutic in the landscape of breast cancer. Of the different combination therapies possible with immunotherapy, few are as unique as focused ultrasound. Magnetic resonance guided focused ultrasound (MRgFUS) is a non-invasive surgery that uses sound waves to thermally damage tissues with high specificity. Like a magnifying

glass converging rays from the sun, focused ultrasound concentrates ultrasound energy to a very small focal region. The amount of thermal damage induced at a focal spot can be monitored and determined using thermometry methods available to magnetic resonance imaging (MRI). It has been shown that MRgFUS exposure on the tumor microenvironment releases inflammatory factors necessary for a systemic immune response.

This process can also lead to recruitment of additional lymphocytes through partial destruction of the tumor and its boundaries. To investigate this, we designed a set of experiments to examine the efficacy of MRgFUS and checkpoint inhibitor immunotherapy in a pre-clinical breast cancer mouse model. In total, six treatment groups were planned for female mice with orthotopically implanted tumors. Four of these groups being treated with light or thorough ultrasound ablation with or without an immunotherapeutic. The other two groups being immunotherapeutic only and an untreated control. Mice were randomly enrolled into one of these groups when their tumors reached a certain size. We then harvested tumors two weeks beginning the of treatment after for immunohistochemistry and cellular composition analysis via flow cytometry. The hypothesized outcome being the combination (light or thorough ablation with immunotherapeutic) treatment would decrease tumor growth rate and increase the

percentage of positive immune factors such as lymphocyte counts compared to the individual treatment controls alone. Our findings should be informative on whether the hypothesis is valid for our model and treatment plan. Additionally, we seek to compare how our results differ from the literature where the effects of focused ultrasound and immunotherapy are interrogated in other preclinical models. The long-term goal of this project aims to eventually compile enough significant evidence to justify a clinical trial in humans.

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## 14. Studying the Manual Control of a Continuum Soft Manipulator

Jacob Hirst (University of Utah)

Faculty Mentor: Haohan Zhang (Mechanical Engineering, University of Utah)

#### Background

Due to their highly flexible structures, continuum manipulators (CM) are uniquely suited for many tasks (e.g., surgery, inspection) beyond the capabilities of traditional manipulators. In my UROP research in Spring 2022, we developed a CM whose shape can be controlled by pulling 3 cables routed through its segments. These cables were controlled by electrical motors with pulleys. Figure 1 shows a picture of the original CM used during the UROP research. Due to the under- actuated nature of this manipulator, controlling its shape automatically

with motors turns out to be a complex mathematical problem. Because of the complexity, we decided to use a data-driven machine learning approach to help solve this. The machine learning library that we used is an open-source python library called BINGO, made by Dr. Jacob Hochhalter and his colleagues at NASA.

#### Problem

The model made to represent the shape of the CM at different motor positions was accurate to our expectations, but the robot is unpredictable in how it goes from one position (or shape) to another. This results in the question – How can we control how the CM moves from point A to point B? To study this, we need to first figure out how we want the CM to be controlled.

#### Methods

The first task was to create a whole new CM. We did this for a couple of reasons. First, the original CM had lots of friction and one broken universal joint, which caused the motion of the CM to be less predictable and fluid. Second, we planned to use VICON to help track the motion of the CM, and there were no permanent mounts for the VICON markers. Figure 2 shows an image of the new vertebrae prototype, which was modeled using Solidworks CAD. The new vertebrae features 3 voids that allow you to insert infrared-reflecting markers that VICON is able to track.

The next task was to create mechanical cranks for humans to be able to control the lengths of the cables that channel through the CM. These cranks were connected to pulleys that retracted and extended the cables when rotated. The cranks were also connected to Qwiic Twist encoders from SparkFun. The encoders tracked the motion of the crank and therefore would allow us to track the lengths of the cables and later study how the CM moved from those lengths. The encoders were programmed to output their data to a CSV when prompted by a PySimpleGUI using Arduino IDE.

Once the new CM and mechanical cranks were developed, we were then able to collect data. VICON was used to track the motion of the CM as 3 volunteers moved the CM from a starting marker to a goal marker using the mechanical cranks discussed earlier. The VICON data and encoder data were synchronized by simultaneously recording a voltage reading from the Arduino pins. Since the frequency of the VICON data being collected was higher than the frequency of the encoders, a high voltage was written to an Arduino analog pin every time that an encoder value was read. This voltage went through a BNC cable, and was written into the VICON data. The VICON and encoder data were synchronized in post-processing using these voltage readings. Pictures of the full data collection setup are shown in Figures 3 and Figure 4. Our goal was to create 3 BINGO models that predict the x, y and z coordinates of the far end of the CM using the encoder values from the mechanical cranks as an input, with a MAE of 5 mm for each degree of freedom.

#### Results

With the synchronized data, we used BINGO to create a model that predicts the X, Y and Z coordinates of the end of the CM with 3 given encoder values. The models had a MAE of 5.25 mm, 11.26 mm, and 1.73 mm for the x, y and z coordinates, respectively. We reached our goal with the z axis, but the x and y axis need more data and more time to learn in order to become more accurate.

#### Conclusion

The bulk of this research project has been completed, and we are proud of the results we have. The BINGO models obviously do not show the MAE we desired, but that is mostly due to a mistake made during the data collections, which limited the size of our datasets. In future work, there needs to be more iterations of the CM, as well as more data collection. This way, we will hopefully be able to create a more accurate BINGO model that can more accurately determine each degree of freedom of the CM.

#### Figures

Figure 1: UROP CM controlled by motors from the research done in Spring 2022



Figure 2: New vertebrae prototype featuring mounts for the VICON markers



Figure 3: Data collection setup with mechanical cranks (front view)



Figure 4: Data collection setup with mechanical cranks (side view)



About the Author

Jacob Hirst

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#### UNIVERSITY OF UTAH

15. Development of a Utah Optrode Array for Large Scale Optogenetics in Non-Human Primate Cortex: Analysis of Spatial Excitation Pattern Using C-FOS

#### Expression

Alexander Ingold (University of Utah); Alessandra Angelucci (Biomedical Engineering, University of Utah); Andrew Clark (University of Utah); Justin Balsor (University of Utah); Frederick Federer; Christopher F. Reiche (University of Utah); and Steve Blair (University of Utah)

Faculty Mentor: Alessandra Angelucci (Biomedical Engineering, University of Utah)

#### Abstract

A central goal in neuroscience is to understand how neural circuits generate the computations that underlie neuronal responses, sensory perception, and behavior. This requires manipulating their activity and determining the results of these manipulations. Optogenetics is a versatile laboratory technique that uses light to manipulate the activity of genetically modified neurons to express exogenous light-sensitive opsin proteins. Optogenetics allows control of neurons on a millisecond time scale and targets specific types of neurons. An optrode, or optical electrode, is a device capable of delivering light to the deep layers of the cerebral cortex that cannot be reached by surface illumination alone. The Utah Optrode Array (UOA) is a waveguide array intended to conduct light to deep cortical layers over a large volume of cortical tissue. Developed by the Blair/Angelucci team at the University of Utah, the UOA is a 4 x 4 mm square array, with one hundred, spike-shaped, glass shanks that pierce below the cortical surface. Here, we

have tested UOAs in non-human primate (NHP) cortex to determine the efficiency of the UOA in delivering optical signals to deep tissue, and the spatial distribution of the neural excitation obtained via optical stimulation through the array. To this purpose, we have used C-fos expression. C-fos is a marker protein that provides an indirect measure of the spatial pattern of neural activation in the brain after immunohistochemical processing. This thesis presents the c-fos related portion of a larger paper incorporating electrophysiological testing data [1]. Our aim in this thesis was to use this protein marker to determine the excitation of neurons induced after using the UOA in macaque monkeys. We found broad- ranging c-fos expression across after UOA stimulation. cortex the visual Glutamate Block, which prevents synaptic transmission, reduced c-fos expression to the neurons directly underneath the UOA. Our results the UOA is effective tool for indicate an optogenetically stimulating a large area of neurons across all cortical layers.

#### Introduction

Optogenetics is a powerful experimental technique that can be used to answer questions about anatomy, physiology, and behavior of neural circuits. It has potential to treat a broad range of diseases such as spinal cord injury [2], multiple sclerosis [3], Alzheimer's disease [4], Parkinson's disease [5], epilepsy [6], and retinitis pigmentosa [7]. Optogenetic techniques are based on the use of light to control the activity of neurons that are genetically modified to express exogenous light-sensitive opsin proteins. The expression of these opsins (light-gated ion channels) in the cells is induced through a viral vector or other gene insertion method. Experimentally manipulating neurons expressing opsins provides many advantages over traditional techniques used in neuroscience. Conventional techniques such as electrical or pharmacological neural manipulation lack cell-type specificity and time precision (acting on a time scale of minutes or hours, while neurons act on a time scale of milliseconds). Optogenetics can be used to excite or inhibit neurons on a millisecond time scale, and it can target specific types of neurons by taking advantage of genetic engineering techniques like viral vector-delivered transgenes [8].

Transgenic mice are easy to generate, but it is more difficult to source transgenic non-human primates (NHPs). Extending optogenetics to non-genetically tractable model species such as NHPs is crucial due to their similarities to human laminar and columnar cortical geometry. NHP studies bring optogenetic technology closer to therapeutic use in humans.

One limitation of optogenetic manipulation of neurons is the inability to target the deep layers of the cortex (about 1.5-2 mm from the surface), because light shone on the cortical surface cannot penetrate deep in brain tissue. However, there is a scientific need

for researchers to be able to manipulate neural circuits involving cells that do not reside in the light-accessible upper layers of the cortex. One solution to this is the use of an optrode. An optrode or optical electrode is a device capable of delivering light to the deep layers of the cortex [9]. Optogenetic experiments in NHP mainly use surface illumination or a penetrating probe consisting of a single optical fiber [2]. A single optrode can only photo activate a small subcortical volume; however, an array of many small diameter fibers could activate a larger subcortical volume. Activation of larger volumes is necessary in NHPs due to their large brain size; relatively large brain volumes must be optogenetically activated to observe measurable behavioral effects.

The Utah Optrode Array (UOA) [9] is a novel waveguide optrode array modeled after the Utah Electrode Array which has been an industry standard used for large scale electrical recording and stimulation of cortical tissue for over three decades [10]. The UOA is a  $4 \times 4$  mm array with one hundred spike-shaped optrodes arranged in a  $10 \times 10$  configuration with customizable length (up to 2.5 mm) and shank width (80-120 µm). This can be inserted directly into the cortex, thereby providing light via each of the 100 shanks to the deeper layers of the brain [11]. Here we have tested UOAs in cortical brain tissue of macaque monkeys to determine the efficacy of the UOA in delivering light to deep tissue, and the spatial distribution of the neural excitation obtained via array photostimulation.

A critical parameter is the spatial extent of the resulting stimulation, i.e., the width and depth of neuronal tissue that can be photoactivated via the UOA. This can be measured both in areas of the brain transfected with light gated ion channels and, in those without opsins as controls. One way to measure such photoactivation is using c-fos

immunohistochemical staining as a tool for determining the extent of neural activation. C- 2

fos is a growth factor protein that is rapidly produced in neurons when they are stressed or activated [12]. Due to the increase in expression levels of c-fos after neural activation, cfos expression can be used as an indirect measure of the spatial pattern of neural activation in the brain post-mortem. Here we used immunohistochemical staining for the c-fos marker protein of neural excitation to determine the spatial excitation pattern induced by the UOA.

#### Background

#### A. Electrophysiology

In 1790 Galvani demonstrated electrical control of nerves. In his public demonstrations, he used electricity to make cadavers sit upright [13]. This inspired fictional works such as "Frankenstein" by Mary Shelley. Electricity causes an action potential by delivering electrical charge to the extracellular space around neurons. This opens the ion channels gated by electric potential which are key in the propagation of the action potential.

Electrical stimulation was applied to the field of visual neuroscience in 1929 by Otfrid Foerster. He found that electrical stimulation of the visual cortex can produce visible points of light in the visual field called phosphenes. Scientists hypothesized that many precisely placed electrodes could be used to create a visual prosthesis [14].

In 2021, Richard Normann from the University of Utah, and Eduardo Fernández from Miguel Hernandez University operated on Berna Gómez a former science teacher who has been blind for 16 years. A 10×10 Utah Electrode Array (UOA) was inserted in her visual cortex for six months providing the ability to see the outlines of objects and doorways. Berna Gómez could even read brail visually. The effectiveness of this visual prosthetic was limited by the number of electrodes and the algorithms controlling the stimulation pattern of the electrodes [15]. The development of algorithms is difficult because it requires the patient to communicate the visual results of each trial to the researcher. A system of many electrodes simultaneously stimulating and recording may be able to avoid the need for the patient to describe the phosphenes they are seeing [15].

Electrode array technology is progressing rapidly with advances in microelectronics. Modern electrode arrays provide large amounts of data through many probes to act as a brain computer interface. The Neuropixel<sup>®</sup> 2.0 is a commonly used probe with up to 6,000 recording sites on one side of a long thin spike. This updated Neuropixel® is a significant advancement over the Neuropixel® 1.0 which was released in 2016 and is used in over 400 labs [16]. Neuralink® is another advanced brain computer interface in development. This interface consists of at least 32 tiny linear arrays with 32 electrodes each robotically placed to avoid blood vessles [17]. Neuralink® reduces damage to the brain and reduces the brain's foreign body response. There is a huge amount of innovation in the field of brain computer interfaces, but there are also many problems that still need to be solved before they can be clinically viable.

The foreign body response is a major challenge for long term implanted devices, especially for implants in the central nervous system. The foreign body response is a series of reactions that protect the body from macroscopic exogenous objects such as brain probes. After implantation, antibodies and other proteins adsorb to the surface of the foreign body. Next, macrophages are attracted to the site to break down or encapsulate the foreign body. The macrophages fuse to form a mass of multinucleated giant cells and fibroblasts produce a fibrous encapsulation of the foreign body. The foreign body response is rapid in the central nervous system [18]. It degrades the performance of electrodes by displacing the neurons near the device increasing the current required for stimulation and increasing the noise in recordings [18].

**B.** Optogenetics History

The history of optogenetics begins in the early 1800's when scientists noticed that a single-celled green algae called Chlamydomonas quickly responds to flashes of light [19]. These algae contain proteins that perform cellular functions when exposed to light. Bacteriorhodopsin was one of the first opsin proteins to be isolated. It was isolated in 1970 from the cell membrane of halobacterium. Bacteriorhodopsin is a light activated proton pump which is not useful for manipulating neurons [19].

In 2003 Channelrhodopsin-2 (ChR2) was discovered [20] following the discovery of the proton pump Channelrhodopsin-1 [21]. ChR2 is a light-gated ion channel which can be used to manipulate neurons in animals. Unlike bacteriorhodopsin, this pump transmits cations which can be used to trigger action potentials in neurons. ChR2 is a commonly used protein in optogenetics [20], [22]–[23]. It is the opsin used in the experiments presented in this thesis [1].

Halorhodopsin is a light-sensitive chloride pump discovered in 2007 [24]. It inhibits neurons by allowing the influx of chloride ions into the neurons. Neurons are hyperpolarizing when yellow light is shone on the cells. This discovery
dramatically increased the capability of optogenetics. This allows for inhibitory and excitatory control of neurons using different colors of light [25].

Optogenetics is a very versatile method of stimulation. The method allows for high temporal precision with frequencies on the order of 100Hz [26]. It is possible to stimulate large areas of cortex by delivering light through a craniotomy or thinned skull. It is also possible to achieve single cell resolution of stimulation using a 2-photon microscope. Cells could be individually stimulated at a depth of 50 microns [27].

The first use of optogenetics to control the behavior of an animal was in 2005. Photo stimulation of the giant fiber system and dopaminergic neurons caused changes in behavior such as wing beating in drosophila (fruit flies) [28]. In 2014 mice were conditioned to avoid hiding in a dark den by using optogenetic techniques to silence dopaminergic neurons. Targeting dopaminergic neurons directly affects the reward systems in the brain. Behavior changes were observed within the first 5 minutes, and mice remained conditioned to stay in the bright portion of the cage for days after the stimulation [29].

C. Optogenetic Methods

Optogenetics requires genetic modification of cells. The two common methods of modification are the use of viral vectors or the generation of transgenic organisms (genetically modified before birth) [30]. This study uses viral vectors carrying genes for opsin protein expression; these are injected into the nervous tissue of interest and allowed to replicate and express their genes until transfection is complete. The type of virus injected determines the pattern of transfected cells. Anterograde vectors infect neurons in the same direction as the action potential (infecting the dendrites or cell body first), while retrograde vectors travel in the opposite direction (infecting the terminal axons first). Trans-synaptic vectors can cross synapses and move between cells.

The expression of optogenetic proteins is usually targeted to specific cell types using one of two methods. The first method is the use of a tissue or cell specific promoter preceding the gene that expresses the opsin protein [31]. The promoter will only initiate the viral gene transcription in the proper cellular environment. For example, CamKII is a promoter that is excitatory neurons in the neocortex specific to and hippocampus [31]. The gene encoding an optogenetic protein is expressed in the cells that recognize the promoter. Another method for targeting a specific cell type is the use of a transgenic organism with Cre recombinase. Cre is an enzyme that can excise or flip the direction of a sequence of DNA between two lox-P sequences [32]. This mechanism can be used to activate a gene encoding an optogenetic protein. A transgenic organism with Cre expressed only in the cells of interest allows targeting optogenetic protein expression to the cells of interest [32].

# **D.** Clinical Applications

Optogenetics has been challenging to apply clinically because it is invasive. For example, some viral vectors can be toxic to the brain causing necrosis of transfected cells. The process of injection can also cause cell death. Optogenetics also requires a method for light delivery which may require a transcranial implant such as an optical fiber or a full implanted lightdelivery device.

Optogenetics has been used to treat spinal cord injury. Stimulation of the spinal cord can restore function by bridging over the injured area. It has additional benefits of triggering cellular regeneration mechanisms, increasing neuroplasticity, and promoting myelination of axons [3]. Optogenetics was used to restore respiratory function in rats following a cervical spinal injury. The phrenic nerve was stimulated restoring respiration and EMG signals on the lesioned side to a level that matched the control side [2].

Optogenetics could be used to treat Multiple Sclerosis (MS), an inflammatory demyelination disease leading to muscle weakness and coordination problems. It is possible to treat MS with neural stimulation which leads to an increase in myelination [3]. This can be done with electrical or optogenetic stimulation. Electrical stimulation may cause more side effects than optogenetic stimulation because it lacks the cell-type specificity allowed bv optogenetics. Stimulation of the premotor cortex resulted with ChR2 in mice in increased myelination and neural activity [3]. This proof-ofconcept study shows promise for treating MS with optogenetics.

Optogenetics could be used to treat Alzheimer's disease (AD). AD results in cognitive decline and is characterized by the build-up of beta-amyloid plaques and neurofibrillary tangles in the brain. It is uncertain whether amnesia caused by AD is linked to the decline of memory storage or memory retrieval. A transgenic mouse model of early AD has been generated, in which mice overexpressed presinillin-1 and an amyloid precursor protein. Optogenetic stimulation of hippocampal memory engram cells in this mouse model resulted in increased memory recall over the control cases. Stimulation caused mice to elicit a previously conditioned response of freezing in response to a blue light. Mice without stimulation did not freeze due to their memory impairment. Additionally, stimulation restored dendritic spine density in the dentate gyrus to control levels [4].

Optogenetics could be used to treat Parkinson's disease (PD). PD is characterized by the degeneration of dopaminergic neurons and results in tremors, loss of coordination, and a shuffling gait. PD can be treated pharmacologically or with deep brain stimulation for cases where pharmacological treatment is ineffective. Deep brain stimulation is an invasive surgery that involves inserting electrodes into deep brain nuclei. Researchers used optogenetics, electrophysiology, and computer modeling to determine that deep brain stimulation causes increased activation of cortical somatostatin neurons, the inhibition of parvalbumin neurons, and the reduction of hyperactivity in pyramidal cells in mice. It is hypothesized that the targeted optogenetic activation of cortical somatostatin neurons may be a less invasive alternative to deep brain stimulation [5].

Optogenetics could be used to treat epilepsy. Epilepsy is characterized by seizures caused by neuron hyperexcitability. It can be treated pharmacologically or with electrical stimulation. Pharmacological approaches often have side effects because the medications are designed to suppress neurological activity. This can lead to brain fog, drowsiness, and nausea. Some patients do not respond to medication at all, leading physicians to try electrical stimulation. The responsive neurostimulation system for epilepsy (RNS® System) is a device in clinical use. It monitors neurons to predict epileptic seizures using an implanted electrode array. It then delivers electrical stimulation to prevent the seizure. The average decrease in seizures was 67% after 1 year, 75% at 2 years, and up to 82% after 3 or more years of using RNS in human [33]. Optogenetic stimulation could be a more effective way of preventing seizures because an inhibitory opsin protein can be used. The use of the inhibitory opsin protein halorhodopsin at the epileptic focus was shown to suppress seizures and reduce the likelihood of future seizures in mouse [8].

Optogenetics could be used to treat retinal diseases. There is a large amount of research in expressing optogenetic proteins in retinal ganglion cells to restore light sensitivity in the retina. In 2021 a case of retinitis pigmentosa was treated using an intraocular injection of a virus encoding ChrimsonR, an excitatory opsin protein. Goggles detect local changes in light intensity and project real time light pulses onto the retina. This is the first case of partial recovery after optogenetic therapy [7].

A limitation with the current generation of optogenetic proteins preventing clinical use is a lack of light sensitivity. Most experiments require high powered lasers to generate threshold level stimulation. It would be ideal if future opsin proteins are sensitive enough to restore retinal function using natural light rather than goggles. Red-shifted variants of opsin proteins can be more sensitive because they require lower energy light [34]. Another goal for future optogenetic protein variants is a faster firing rate.

# The ChETA light gated ion channel developed in 2010 can depolarize at up to 200Hz [35].

E. C-fos Overview

C-fos is a proto-oncogene that encodes the protein for a nuclear phosphoprotein. It is a growth factor that is produced after a cell undergoes a variety of stresses. Neurons express c-fos when they have an increased rate of action potentials or if they are physically or chemically stressed [36]. Quantifying c-fos expression using immunohistochemistry can be used to indirectly measure the spatial pattern of neural activation in the brain post- mortem. This is due to the increase in expression levels of c-fos after neural activation. The neural activation can come from a wide range of stimuli such as manipulation, optical manipulation, electrical physical perturbation of the brain, water stress, fear, odors, injected seizure-inducing agents, or even vocalizations of the same species [36]-[40]

Neurons under the optrode array will be directly optogenetically stimulated or indirectly stimulated via synaptic optogenetically activated transmission from neurons. Glutamate is the most common excitatory neural transmitter in the central nervous system. To differentiate direct from indirect activation, in our study we used a Glutamate Block drug to block neural transmission and indirect neural activation. The drug, NBQX, is a selective competitive agonist for the AMPA glutamate receptor. Blocking the AMPA receptor can significantly reduce the transmission of signals between neurons. This reduces secondary neural excitation or the excitation of secondary neurons by primary neurons that are directly stimulated [41].

A key consideration for designing an experiment

using c-fos is that it has a definite refractory period or delayed peak expression. The temporal expression pattern of c-fos after a stimulus has a peak of 1.5 hours after the initial stimulation and has an expression tail that lasts around 12 hours [36]. For this reason, it is important to sacrifice the animal 1.5 hours after stimulation. Since we are working in the visual and supplementary motor (SMA) cortex, it is also important to close the eyes and avoid touching the subject for 12 hours before sacrificing the animal. This minimizes unintended stimulation not caused by the optrode insertion or light stimulation.

The distribution of c-fos expression is visualized post-mortem with immunohistochemical (IHC) staining of the thinly sectioned brain with an antibody against the c-fos protein. The IHC stain (c-fos+ cells) is analyzed by manually counting the cell bodies and empirically determining the distribution pattern of the cells with respect to the stimulation site.

A. General Overview

## Methods

A virus containing genetic instructions encoding for the channelrhodopsin protein was injected into the cortex (Fig. 1). Two weeks later the Utah Optrode Array (UOA) was inserted. A laser was aimed at the back of the array to stimulate the cortex. After the c-fos refractory period of 90 minutes, the animal was sacrificed and perfused. The brain was sectioned, immunostained for c-fos, counterstained for Nissl (to reveal cortical layers), and imaged. The distribution of c-fos was analyzed with manual cell counting.



Fig. 1 (A) Diagram of channelrhodopsin injection. In 3 cases, channelrhodopsin was injected into the cortex through a craniotomy, and left for 2 weeks to allow for suitable expression. (B) Diagram of optrode insertion. A Utah Optrode Array was inserted through the craniotomy into the cortex over the area containing the channelrhodopsin-expressing neurons.

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B. Design

Optrodes were tested in four experimental conditions in three macaques (Macaca Fascicularis) (see Table 1). All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of Utah and conformed to the guidelines set forth by the USDA and NIH. One animal had one of two different experimental conditions performed separately in each brain hemisphere. Case 1 was a UOA with channelrhodopsin transfection, UOA insertion, and laser stimulation in visual cortex. In Case 2, a Glutamate Block of NBQX was applied before UOA insertion over channelrhodopsin transfected SMA cortex and followed by laser stimulation. In case 3, a UOA was inserted into SMA cortex (not expressing any opsins) as a control, to determine whether UOA Insertion-only (with no light stimulation) causes

c-fos expression. Case 4 was another control case which received Light-only stimulation of the SMA cortex (not expressing opsins) with no UOA inserted in cortex.

| Name              | UOA Activation | Glutamate<br>Block | Insertion-only | Light-only |
|-------------------|----------------|--------------------|----------------|------------|
| Channelrhodopsin  | Yes            | Yes                | No             | No         |
| UOA Insertion     | Yes            | Yes                | Yes            | No         |
| Laser Stimulation | Yes            | Yes                | No             | Yes        |
| NBQX              | No             | Yes                | No             | No         |

Table 1: Organization of the four experimental cases.

C. First Surgery for viral injections

Surgery for the transfection of channelrhodopsin was performed in the UOA Activation and Glutamate Block cases. Animals were pre-anesthetized with ketamine (10 mg/kg, intramuscular). An intravenous (i.v.) catheter was inserted, and the animals were intubated with an endotracheal tube, placed stereotaxic apparatus, and artificially ventilated. in а Anesthesia was maintained with isoflurane (1-2.5%) in 100% oxygen, and end- tidal CO2, blood oxygenation level, electrocardiogram, and body temperature were monitored continuously. I.V. fluids were delivered at a rate of 5 ml/kg-hr. The scalp was incised, a large craniotomy and durotomy was made to expose the lunate sulcus, area V2 and parts of V1, or a similar sized craniotomy over SMA cortex. On completion of the craniotomy, isoflurane was turned off and anesthesia was maintained with sufertanil citrate  $(5-10 \mu g/kg/h, i.v.)$ .

We injected a 1:1 viral mixture of AAV9.CamKII.4.Cre.SV40 and AAV9.CAG.Flex.ChR2.tdTomato (Addgene Catalog #s:657 105558, and 18917, respectively). This is an anterograde vector which leads to nearly no retrograde expression [42]. Injections were performed using a picospritzer (World Precision Instruments, FL., USA) and glass pipettes with 35-45  $\mu$ m tip diameter slowly at around 15 nL/min. The mixture was pressure injected; 250-350 nL at 2-3 cortical depths between 0.5 and 1.5 mm from the surface. Two boluses were delivered at 1.1 mm deep and two were delivered at 0.6 mm deep. The pipette was left in the brain after injection 5-10 min to prevent backflow while retracting. Five to six injections of viral mixture made 1.5-2 mm apart each with 500- 750 nL in each injection.

On completion of the injections in the appropriate brain area, a synthetic silicone- based film serving as an artificial dura was placed on the cortex. The native dura was sutured over the artificial dura. The craniotomy was filled with surgical gelfoam and sealed with a piece of sterilized parafilm and dental acrylic. The skin was sutured, and the animal was recovered from anesthesia.

D. Terminal Surgery for optogenetic stimulation

About 5-10 weeks post-injection, the animal was prepared for a second surgical procedure and anesthetized with isoflurane as described above. First, the eyes of the animals were closed for 12-14 hours to ensure baseline c-fos levels. In two of the three animals, craniotomies and durotomies were made over the SMA cortex. The scalp was reincised at the same site as the prior incision, and the artificial dura was removed. Gelfoam was used to protect the cortex and keep it moist. In one case, a Glutamate Block drug (NBQX, 5mM) (Tocris BioSciences, Minneapolis, NM) was applied by soaking a piece of gelfoam with 1mL of NBQX and adding around 0.2 mL every 15 min to ensure saturation. The NBQX soaked gelfoam was applied to the site expressing ChR2 for 90 min. UOAs were inserted into the brain using a pneumatic hammer (Blackrock Microsystems, Salt Lake City, UT, USA) at 20-30 psi with a 1 mm-long inserter. This resulted in partial insertion, so the UOA was gently pushed down to complete insertion. A hole slightly smaller than the optrode was cut in blackened artificial dura. The black artificial dura was positioned with the hole over the UOA to prevent laser light spillover and limit all stimulation to light coming out the tips of optrode shanks.

A 473 nm benchtop laser (400 µm multimode optic fiber, ThorLabs Newton, NJ; laser: Laserwave, Beijing, China) was used to stimulate the optrodes with a measured 38 mW output with 15-19 mW/ mm2. A collimating lens (ThorLabs, Newton, NJ) restricted the spot size to about 1.5 mm in diameter. The estimated threshold of ChR2 is 1 mW/mm2. The optrodes were stimulated in two places on the optrode twice for 2.5 min at 50Hz and twice 2.5 min at 20Hz with a 2.5 min break between stimulations. The laser was moved between stimulation to alternate between the two positions on the optrode. The laser pulse duration was 10 ms with 10 ms or 40 ms between pulses. The optrode was stimulated for a total of 10 mins. The light-only control was stimulated for the full 10 min in one location. After stimulation, the craniotomies were again covered with sterilized parafilm and dental acrylic. The animal was sacrificed 90 min. after the end of stimulation using Beuthanasia (0.22 ml/kg, i.v.) and perfused transcardially with saline for 2–3 min to clear the brain of blood, followed by 4% paraformaldehyde (PFA) in 0.1M phosphate buffer for 20 min to fix the brain.

E. Histology

The brain was postfixed overnight in 4% PFA. It was stored in cryoprotectant at  $-20^{\circ}$  C until ready for sectioning. The brain was cut into a block to isolate the visual cortex or SMA cortex. The blocks were immersed in 30% sucrose for cryoprotection until they became saturated and sunk, then sectioned in the sagittal direction on a freezing microtome at 40 microns thick. All cases were cut sagitally (the plane separating the left and right sides of the body).

Sections were rinsed then put in a blocking buffer of 2% normal donkey antiserum (AB\_2337258; Jackson ImmunoResearch, PA, USA), 2% gelatin from cold water fish skin (Millipore Sigma, MA, USA), 1% Triton x-100 (Sigma Aldrich, MA, USA), phosphate buffer solution for two hours. The sections were moved to primary antibody overnight at room temperature (1:500 rabbit antic-fos ab190289; Abcam, MA, USA). Sections were then rinsed and put in near-infrared fluorescent protein linked to a secondary antibody for 2 hours (1:200 donkey anti-rabbit IgG-AF647; Jackson ImmunoResearch, PA, USA). Sections were rinsed and wet mounted on glass microscope slides.

Sections were stained for Nissl (1:100 Blue Fluorescent Nissl N21479; Thermo Fisher Scientific, MA, USA) to reveal the cortical layers by dripping the solution directly onto the tissue sections on the microscope slides. They were rinsed, blocked for 10 min, stained for Nissl for 20 min adding more dye every 5 min, blocked again for 10 min, rinsed, cover slipped, and double sealed with CoverGripTM coverslip sealant (Biodium, CA, USA).

F . Imaging

Imaging was performed on a Zeiss Axio Imager.Z2 fluorescent microscope using a 10X objective and an Axiocam 506 mono camera (Zeiss GmbH, Germany) with a Zeiss X-cite 120 LED Boost light source. Image files were created and analyzed using Zen 2.6 Blue Software (Zeiss, Germany). The light intensity was set to 100%, and the exposure time for each channel was kept the same between images. Images were collected in channels simultaneously. One three channel observed tdTomato fluorophore attached to the channelrhodopsin protein, and the second channel observed the Alexa Fluor 647 histologically attached to c-fos protein. The third channel observed the Nissl blue 435/455 stain of all neurons to reveal layers.

G. Analysis

Cells were counted manually in Neurolucida

(MicroBrightField Bioscience, VT, USA) and analyzed in Neurolucida Explorer 2006. Regions of interest (ROIs) were drawn at 3 distances from the insertion site, each 200 microns wide and the full depth of the cortex. The first ROI was as close as possible to the center of the stimulation site and/ or UOA insertion while avoiding damage caused by the UOA or injection pipette. The other two regions were 4,000 microns and 8,000 microns from the stimulation site. Five sections from each case were quantified for a total of 15 ROIs per case (5 sections x 3 ROI). The laminar distribution of c-fos was analyzed by tracing the layers visible in Nissl stain and calculating the number of cells within each layer using Neurolucida Explorer. Cell counts were plotted with error bars of 1 standard deviation using Excel (Microsoft) (Fig. 5) and the laminar distribution was displayed using a percentage stacked bar graph (Fig. 6). Other qualitative analysis is made through observation of images. Statistical analysis utilized a one-way ANOVA with post-hoc comparisons and Bonferroni correction to compare experimental and control cases as well as across distances.

# II. Results

We analyzed patterns of c-fos expression using IHC in two control and two experimental hemispheres from 3 animals. In each case we counted the number of c-fos + cells in 3 ROIs located at the site of UOA insertion and/or light activation, and 4 and 8 mm away from it, respectively (see Methods).

Optogenetic activation of ChR2-expressing the primary visual area (V1) via the UOA (Fig. 2 A-D) in the experimental case revealed local and longrange c-fos expression extending far beyond the site of UOA insertion in V1, visible across all layers of V1 and extending to other areas including the secondary visual area (V2) and extra striate cortical areas known to receive inputs from V1 (Fig. 3). V1 shows a bright band of c-fos expression in layer 4C that terminates at the V1/V2 border, revealing this border. The injection leads to the expression of ChR2 light-gated ion channels and tdTomato fluorophore (the latter allows us to visualize the spread of the ChR2 expression at the injected site). The emission color of the tdTomato fluorophore, which is normally red, is displayed in green for purpose of illustration. This extensive pattern of cfos+ cells in this case suggested c-fos expression was induced by both direct neural activation by light as well as indirect activation via synaptic activity.

To test this hypothesis and to determine the extent of neural activation directly induced by UOA photostimulation, we blocked glutamate transmission by applying the AMPA receptor antagonist NBQX to the cortex expressing ChR2, prior to UOA insertion and photostimulation (Fig. 2E-H). This blocks indirect neural activation, thus revealing the neurons directly stimulated by the UOA. The Glutamate Block reveals neural excitation and c-fos expression localized to the volume of cortex underneath the UOA. In this case, we found reduced c-fos expression by 5-fold in the area of UOA stimulation, and by 200- fold at distances of 4,000 and 8,000 microns (Fig. 2E-H, 4).

The control with UOA Insertion-Only (Fig. 2I, 4) showed localized c-fos expression at the site of insertion and tissue damage from the optrode array spikes. This case did not receive ChR2 injection or photo stimulation. The animal was sacrificed 4 hours after UOA insertion in SMA cortex. The image shows the c-fos stain alone, to best demonstrate the optrode spike damage. The UOA in this case was fully inserted with spikes extending to layer 5. UOA insertion-only and the Glutamate Block had similar levels of c- fos expression, but the insertion-only case had greater long-range expression suggesting that mechanical stimulation trans-synaptic secondary neural mav cause activation.

The Light-Only control (Fig. 2K-L, 5), in which the fiber-coupled laser with a collimating lens was directed at non-opsin expressing SMA cortex, showed low levels of c-fos expression localized mostly to layer 1 within 1 mm of the stimulation site.

The blue Nissl stain labels the rough endoplasmic reticulum of every cell and is used to identify the

cortical layers, allowing for determination of c-fos cell counts in each layer (Fig. 2A, 2E, 5). The laminar distribution of c-fos expression reveals a strong bias towards topical layer 1 cells in the Light-only case.

Statistical analysis (one-way ANOVA with pairwise comparisons and Bonferroni correction for multiple comparisons) revealed a significant difference in the number of c- fos+ cells at each distance between the UOA Activation experimental case and the Glutamate Block case (Glutamate Block; p<0.001, at all distances) as well as between UOA Activation experimental case and each of the control cases (Insertion-Only: p<0.001, at all distances; Light-Only: p<0.001, at all distances). There was no significant difference between the Glutamate Block and UOA-Insertion-only cases at any distance (p=1 at 0 mm, p=0.23 at 4 mm, and p=0.44 at 8 mm distance), but both cases differed significantly from the light-only case at 0mm distance (p<0.05 for all comparisons). Finally, the number of c-fos+ cells decreased significantly with distance for cases UOA Activation (p<0.001), Glutamate Block (p=0.001), and Insertion-only (p=0.003), but not for case Light-Only (p=0.079)(Fig. 4).



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Fig 2. (A-C) Experimental Case.

The same sagittal section encompassing parts of V1 and V2 is shown under 3 different fluorescent illuminations, to reveal Nissl stain (A), tdT/Chr2 expression (B; the red tdT fluorescence was converted to green for purpose of illustration), and c-fos IHC (C). White solid contour: V1/V2 border; dashed contours: layer boundaries (layers are indicated); white boxes: ROIs

(numbered 1-3 in panel C) where c-fos+ cells were counted. Asterisks in (B) mark the core of the viral injections, and sites of highest tdT/ChR2 expression. P: posterior; V: ventral. C-fos expression in this case is observed throughout all layers (local) and across cortical areas (long-range). Scale bar in (A): 1mm (valid for A-C). (B,F) White arrows indicate the visible damage caused by each UOA needle, while the gray arrow points to the likely location of one of the UOA needles which did not cause visible damage in this section. (D) Higher magnification of c-fos IHC in and around each ROI. Scale bar: 0.2mm. (E-H) Glutamate Block. Same as in (A-D) but for the Glutamate Block case in which an AMPA receptor antagonist was injected into the SMA prior to UOA insertion and photostimulation. The sagittal section is from the SMA. D: dorsal; A: anterior. Scale bars: 1mm (E and valid for E-G); 0.2 mmm (H). Blocking AMPA receptors demonstrates that initial optogenetic activation is limited to the stimulated layers in the region of UOA insertion. (I-J) Insertion Only. C-fos IHC in a sagittal section of SMA cortex (I) and at higher magnification in and around each ROI used for cell counts (J), in a case which only received UOA insertion. Scale bars: 1mm (I), 0.2mm (J). (K-L) Light Only. Same as in (I-J), but for a control case in which SMA cortex only received surface photostimulation via an optical fiber-coupled laser. Here only one ROI is shown at higher magnification to reveal the few labeled cells in L1. Scale bars: 0.5mm (K), 0.2mm (L).



Fig. 3 Large tiled sagittal image showing long range expression of c-fos protein in orange. Arrows indicate the border between V1 and V2. Scale bar: 4mm top right. The weakened tissue near the optrode insertion site was damaged while sectioning the tissue.



Fig. 4. Average c-fos cell count at varying distances from stimulation site (mean +/- stdev, n=5).



Fig. 5. Laminar distribution of c-fos counts reveals the depth of c-fos expression. The experimental case contains the least expression in layer 1. Glutamate Block and Insertion- only contain more topical expression, while Light-only contains primarily topical expression.

### Discussion

We tested the Utah Optrode Arrays (UOAs) in

macaques to determine the efficiency of the UOA in delivering light to deep tissue and the extent of the spatial distribution of neural excitation obtained via optrode array photostimulation. C-fos staining of stimulated tissue revealed long-range excitation across the entire visual cortex in the experimental condition (Fig. 2 A-D & Fig. 4). This long-range excitation is interesting because the UOA was designed to deliver light to the deep layers directly below the insertion point [9]. Our results indicate that the UOA may be useful to investigate interareal network interactions in the brain.

Glutamate Block significantly reduced long range likely due to reduced synaptic excitation, transmission between neurons. There was a 4.5-fold reduction of c-fos expression at the optrode insertion site and a 200-fold reduction 4000nm and 8000nm from the UOA insertion site (Fig. 2E-H & Fig 4). The NBOX Glutamate Block performed as predicted and established that the stimulation directly from the UOA was localized to the cortex UOA. Electrophysiological underneath the recordings in ChR2-expressing V1 cortex photostimulated via a UOA demonstrated that spiking was not observed beyond about 3mm from the stimulated site [1]. Therefore, c-fos expression observed far from the stimulation site reveals subthreshold neural activity. Subthreshold c-fos activity is consistent with other work such as the use of c-fos for metabolic mapping of the brain [43].

An important control was to determine whether c-fos expression underneath the UOA could result only from light stimulation or mechanical stimulation from the insertion of the UOA. The Insertion-only case represents one such control case in which the UOA was inserted into the cortex which did not receive channelrhodopsin injections or light stimulation. While there was some c-fos expression underneath the optrode array in the Insertion-only control, there were five times more activated cells in the experimental case. In comparing the controls with each other and the experimental case, there was slightly more c-fos expression from the Insertiononly case compared to the Glutamate Block case (Fig. 4). This did not negate any of the findings, instead it demonstrated the significant reduction secondary excitation compared with the in experimental case that include UOA activation with channelrhodopsin injection. The control using Light-only stimulation of non-virally transfected cortex revealed low levels of localized expression confined primarily to layer one, the most superficial layer (Fig. 4, 5). This reveals a very low baseline of c-fos expression even when the cortex has been exposed to light.

One potential limitation to this paper is the fact that the experimental case was performed in the visual cortex, while the control trials and Glutamate Block were performed in the SMA cortex. This somewhat limits our knowledge of how c-fos expression in visual cortex compares to its expression in SMA cortex. It would be beneficial to perform another experimental trial in the SMA cortex. This would also provide valuable information comparing the excitability of c-fos in SMA and visual cortices. However, for ethical considerations and to keep the number of animals small, these experiments have all been done in animals that already served important research purposes in other additional studies.

A common method of optical stimulation is aiming a laser directly at the cortex rather than aiming it at the back of an optrode array [4]. It would be informative to perform a control case with direct light stimulation to cortex that is expressing channelrhodopsin protein. This would allow for comparison between traditional methods and the optrode array on c-fos expression. Such a control was performed in the submitted paper [1] using electrophysiological recordings in response to surface and UOA photostimulation. It was found that surface stimulation leads to superficial layer activation, while activation via the UOA leads to deep layer neuronal activation. Another control experiment should be performed in the future applying Glutamate Block drug to blank cortex before inserting the UOA. This would determine how much of the c-fos expression in the Glutamate from optogenetic Block comes light case stimulation and how much comes from the UOA Insertion-only.

The UOA has new variants such as the Active

Array which has a  $\mu$ LED array bonded to the backplane of the UOA. It is capable of delivering light directly to the individual UOA shanks without using a separate laser for stimulation [11]. This allows the researcher to stimulate any combination of small regions below each optrode shank. Further research could investigate stimulation patterns of this new UOA type.

Many labs are developing devices that combine optical stimulation with electrical stimulation and recording. This is very convenient because researchers can skip the step of inserting a separate recording array to measure the electrical response to optogenetic stimulation. The UOA used in this experiment does not have electrical recording capabilities, but the lab that is developing the UOA is also working on a device with optrode shanks as well as recording electrodes [46]. Various production methods have been used to create devices for optogenetic stimulation and electrical recording such as a high- density CMOS surface array, a coaxial style optical/electrical probe, a 4×4 optical array with 5 recording channels, and a 1024-channel penetrating electrode array with two optical fibers [47]-[50]. These devices are useful for characterizing the electrical response to stimulation, so optogenetic labs many are developing this type of array.

There are interesting concepts for optogenetic stimulation-only devices. Each concept has an ideal

application. A high-density liquid crystal surface stimulation device has the potential to scale up to millions of channels [51]. This is good for stimulating large areas of the surface of the brain, but the precision implied by the pixel density may be deceiving. The problem with using high spatial density of optrodes such as the liquid crystal array or the UOA is that light tends to diffuse in the brain and stimulate more volume than what is intended. Laser light may diffuse several millimeters in all directions [52]. This makes the adjacent optrodes redundant. There is a limit to the precision of optrodes. Fortunately, single cell resolution in optogenetic stimulation has been achieved using a 2-photon microscope [27]. A 2-photon microscope is challenging to use, bulky, and expensive, but it is much more precise than an optrode. The advantage of the UOA is not its precision. As shown in this paper, advantage is in its ability to stimulate a large subsurface volume. The UOA is the only device with such a large array of surface penetrating optrodes. This is perfect for activating large volumes of subsurface neurons. This is supported by the c-fos distribution observed in this paper (Fig. 3-4).

This thesis characterizes the c-fos response from stimulation with the UOA. There are no other pulibcations that characterize the c-fos response of an optrode array. The results from the UOA align with results in other papers in that optogenetic activation leads to c-fos expression [37]–[39], [41], [44], [45], [53]. This paper differs because UOA delivers light to a relatively large cortical volume compared with surface stimulation or single fiber stimulation. This has resulted in c-fos expression at distances far from the stimulation site that exceed distances observed in other papers [43], [44].

The long-range effects of optogenetic stimulation observed using the methods discussed in this paper are a significant discovery. This provides a method to influence many neurons in a safe and effective way. The number of neurons affected by electrical stimulation is dependent on voltage, so stimulating large numbers of neurons would require a high voltage or many electrodes spread across a large area. Optogenetic stimulation using an array of optrodes could be used for treating epilepsy, Parkinson's disease, and other neurological diseases that may respond to the therapeutic manipulation of large numbers of cortical neurons.

The mapping of the stimulation pattern under the UOA is an important step in the development of this device. Optogenetics has a huge potential because it can inhibit and excite neurons and target specific cell types. Its ability to stimulate cells with greater temporal precision than electrical stimulation [8] may be extremely useful for improving brain machine interfaces and developing prosthetic vision. The future is bright for optogenetics.

## Acknowledgements

Support from the National Institutes of Health is acknowledged. Dr. Alessandra Angelucci is the lab principal investigator who supervised the thesis. Dr. Andrew Clark oversees electrophysiological and optogenetic aspects of the thesis and the corresponding preprint paper. Frederick Federer oversees surgical aspects of these experiments. Dr. Justin Balsor is a post-doctoral researcher who acts as a mentor in histology, microscopy, and data analysis. Dr. Steve Blair Lab designs and manufactures the Utah Optrode Array.

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# 16. Making Smart

# **Hospitals Useful**

Thomas Kauffman (University of Utah) and Joshua Dawson (University of Utah)

Faculty Mentor: Jason Wiese (School of Computing, University of Utah)

Smart hospitals are arriving, driven by the vision to enhance the patient experience, reduce operational burden, and improve hospital workflow. The University of Utah's newly constructed Craig H. Neilsen Rehabilitation Hospital contains patient rooms where the lights, blinds, thermostat, TV, and wireless soundbar are all controlled through an app on a hospitalfurnished iPad or personal device. This novel implementation supports varying control abilities through touch, voice command, sip and puff controller, or physical switches and remotes. This technology is potentially transformative for patients experiencing motor or mobility impairments, helping them regain lost freedom and control of their surroundings. We explore how the technology employed in patient rooms affects – and can better support –patients' and other stakeholders' needs and experiences, how the smart room technology fits in the context of a hospital setting, and how the patient's experience with the technology affects how they view their transition to home through semi-structured user study interviews. We identify a range of considerations that inform the way smart technology is integrated into hospital environments, including design decisions about the technology itself, but also adjustments to the way that hospital staff introduce and support the technology to patients. Through continuing work, we can guide future designers in seamlessly integrating technology into the hospital environment to reduce burdens on all stakeholders, support patients' unique physical abilities, and enhance independence for those who have lost it.

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17. Investigating

### **Curvature Induced**

## Circumferential

### **Drifts to Improve**

## the 3D

## **Reconstruction of a**

# **Deployed Stent**

Michael Keyser (University of Utah)

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### Introduction

Coronary Heart Disease (CHD) is one of the most common causes of death in the United States. It accounts for 370,000 deaths annually. Various lifestyle choices, such as maintaining a healthy diet, exercising, not smoking, and limiting alcohol consumption to moderate amounts reduce the risk of CHD, but sometimes proper lifestyle choices are not sufficient [1]. CHD is caused by a buildup of plaque in the arteries called atherosclerosis. The plaque blocks blood flow to the myocardial tissue, which can lead to a heart attack. Clinicians use stents to open arteries blocked by plaque to restore blood flow in the affected artery. Stenting arteries is generally successful, but there is up to a 30% chance the stent will fail due to the ingrowth of tissue that re- blocks the vessel – termed restenosis [2].

Biomechanics has been shown to play a key role in stent failure [3]. Currently, studies that investigate the biomechanics in stented regions are limited to general cases because they lack the ability to model the in vivo geometry of the stented region. Elliott et al. developed a technique that utilizes optical coherence tomography (OCT) imaging data to reconstruct the in vivo deployed stent and vessel geometry [4].

In summary, the stent struts are reliably identified in OCT images [5]. Through fusion with biplane angiography [6], the stent struts are matched to the shape of the vessel to create a sparse representation of the known locations of the stent after being deployed, herein OCT point cloud. The physical dimensions of the stent are known, so an accurate model of the stent can be constructed and fitted to the OCT point cloud, through constrained deformation process called а diffeomorphic mapping [4]. Once the diffeomorphic mapping is complete, an accurate geometry of the patient specific stent has been reconstructed and the biomechanics in the region can be studied. The accuracy of the OCT point cloud is integral to the process because the diffeomorphic mapping assumes the OCT point cloud is correct.

A rotational shift was observed in the collected OCT images. The curvature of the vessel caused the drift in OCT images. This imaging artifact is referred to as circumferential drift. OCT images can be adjusted prior to strut identification to correct for the effects of circumferential drift. An experimental testbed with three channels of constant curvature were scanned and the circumferential drift was measured across the OCT stack. The circumferential drift was observed in the direction of the curve. The normal vector of a curve in the Frenet-Serret frame (TNB) points inwards on a curve. Finally, the Frenet-Serret frame (TNB frame) was calculated at each point along the centerline to determine the direction of circumferential drift.

### Methods

A. Algorithm for Identifying OCT Image Angle

Three separate channels were drilled out of a Deldrin® experimental test bed using a CNC- machine. The three separate channels had curvatures of  $\kappa = 20$ -1mm, 30-1mm, and 60-1mm, respectively. The channels were drilled to appear as a 'U' when viewing the OCT images in Cartesian. An OCT catheter (DragonflyTM OPTISTM, Abbott Vascular) was inserted into each channel to acquire OCT images. The channels were scanned in both "high-resolution" and "survey" mode where the distance between OCT frames is 0.1 mm and 0.2 mm, respectively.

The top of the 'U' was identified and its angular change across images was tracked. Figure 1 shows there is a faint linear section along the top of the 'U'. The angle of the faint section is the angle of the OCT frame.



Figure 1. OCT image of experimental testbed in Cartesian.





The points making up the faint line were identified by analyzing the OCT image in its polar and Cartesian views. First, the OCT image was viewed as a polar image. Each OCT image has 496 axial lines (A-lines) and a depth of 984 pixels per image as shown in figure 2. The peak- intensity depth was identified in each A-line and was filtered with intensities greater than 25% of the mean intensity were removed. Noise was removed by filtering out depths where intensities changed by more than 5 between adjacent depths. Intensities were then filtered to consider depths within 20 below the median intensity and one standard deviation above the mean intensity. The remaining depths consisted of the faint linear section along the top of the 'U'.

The OCT image was converted to Cartesian where the faint section appears linear. A line was fit through the points. Outliers were removed and a new line of best fit was calculated up to three times. The leftmost and rightmost of the remaining points were chosen to calculate the angle of a line passing through the points. Figure 3 shows the line used to compute the angle of the OCT image.



Figure 3. OCT image with the selected line shown in yellow. B. Calculating Circumferential Drift

The angles of the OCT images were plotted against the position in the OCT stack as shown in figure 4.



Figure 4. Image angle plotted across the OCT stack.

Angles were filtered using a Gaussian filter. Noise created by random rotation was present in many of the measurements due to the imaging probe rubbing against the channel, which is common in OCT scans. A region without substantial noise was selected and its corresponding set of OCT images were inspected to confirm a smooth rotation. The smooth region had an approximately linear slope. A line was fit through the region and the slope of the line was the circumferential drift per frame. The drift per frame was multiplied by the number of frames in a millimeter (10 frames/mm for "highresolution", 5 frames/mm for "survey" mode) to get the circumferential drift per mm. The average of the circumferential drift per mm in the "highresolution" and "survey" modes was reported as the circumferential drift per mm.

Tracking the angular change of the 'U' quantified the magnitude of the circumferential drift but did not account for the direction of the circumferential drift. OCT images were manually reviewed, and the circumferential drift was observed towards the inside of the curve. Each OCT scan is accompanied by a biplane angiogram that provides 3D centerline data for the OCT stack OCT images were arranged in 3D space along the centerline as shown in figure 5. The TNB framework was calculated for each OCT frame along the centerline. The normal vector points inwards on the curve (i.e., in the direction of circumferential drift) and calculating the angle between the normal vector and the vertical of the image quantifies the direction of the OCT circumferential drift. If the normal vector is to the left of the vertical, the circumferential drift is counterclockwise. The normal vector right of the vertical indicated the circumferential drift was clockwise.



Figure 5. OCT images properly aligned in 3D space using the centerline from an angiogram.

C. Correcting the Full Stent Reconstruction to Account for Circumferential Drifts

А circumferential drift correction was implemented into the method developed by Elliot et al. [4]. First, the circumferential drift of each OCT image was calculated independent of the stent reconstruction The cumulative process. circumferential drift was calculated for each image by summing the drifts of all preceding images in the stack. Drifts could be negative due to their direction and summing preceding drifts accounts for changes in drift direction. Images were adjusted for drift by circularly shifting the A-lines in the polar image (circular shift means A- lines "wrap-around" when shifted, e.g. A-line 496 will move to A-line 1 when shifting down 1). Shifting the A-lines in a polar OCT image are equivalent to rotating the OCT image where one A-line is equivalent to 0.726°. Shifting A-lines was chosen over rotation in Cartesian to avoid changing pixel values from interpolation present in image rotation algorithms. Pixel values must not be altered to not affect the performance of the stent strut identification algorithm. Once all images in the OCT stack were adjusted, stent strut identification could begin.

### Results

A. Formula Relating Curvature to Circumferential Drift

The circumferential drift was found to be -0.1719°/mm, -0.5979°/mm, -0.9727°/mm, and – 1.1243°/mm for curvatures of 0, 1/20, 1/30, and 1/60 mm-1, respectively. The straight channel (curvature of 0 mm-1) had a drift of -0.1719°/mm because of catheter movement, which is an acceptable noise level. Figure 6 shows the drifts were plotted against curvature and the line of best fit gave the formula relating curvature to circumferential drift in degrees per millimeter where  $\kappa$  is curvature and  $\theta$  is the circumferential drift. Equation 1 is the line of best fit and the formula relating curvature to circumferential drift.



Figure 6. Plot showing the linear relationship between curvature and circumferential drift.

♦ = -19.12♦ - 0.227

Equation 1. Relationship between curvature and circumferential drift.

B. Validating Angle Correctness Algorithm

The angle identification algorithm performance was compared to a 'gold standard' set by manual inspection. 50 OCT images were randomly chosen from two OCT scans (50 from each scan) and were manually inspected. The angle of each image was manually calculated using the Angle Tool in ImageJ. A Bland-Altman plot and Lin's concordance correlation coefficient (CCC) showed a strong agreement between the algorithm and the 'gold standard.' Figure 7 shows there is no systematic difference between the algorithm and 'gold standard.' The CCC was 0.99103, showing there is strong agreement between the algorithm and 'gold standard.'



Figure 7. Bland-Altman and CCC plots validating the results of the angle detecting algorithm.

### Conclusion

Existing stent reconstructions suffer from curvature induced circumferential drifts. We successfully developed a framework the magnitude that quantified and direction of the circumferential drift and adjusted OCT images to counteract the effects of the circumferential drift. The circumferential drift was found to be -0.1719°/mm, -0.5979°/mm, -0.9727°/mm, and - 1.1243°/mm for curvatures of 0, 1/20, 1/30, and 1/60 mm-1, respectively. Linear regression was used to relate curvature to circumferential drift given by equation 1. Future work will investigate the effects of circumferential drift corrections on the completed 3D stent reconstruction.

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About the Author

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### 18. Online

### Learning with

### **Spiking Neural**

### **Networks for Data**

### Prefetching

James McMahon (University of Utah)

Faculty Mentor: Rajeev Balasubramonian (School of Computing, University of Utah)

The focus of our work was in creating a Data Prefetcher that uses a Spiking Neural Network as its primary means for generating predictions. We have proposed two different implementations, each with their own strengths and weaknesses. One implementation utilizes a singular Spiking Neural Network in order to make its predictions, and the

second implementation uses multiple separate networks divided along program counter and page pairs that exist in memory accesses. The singular network model is a more easily implementable prefetcher, and the multi-network model is far more accurate in the predictions that it makes, but suffers from low coverage. In terms of performance (measured as instructions per cycle or IPC), the baseline Best-Offset prefetcher, our single-network prefetcher, and our multi-network prefetcher achieved IPCs of 0.528, 0.483, and 0.463 respectively. In terms of accuracy, the numbers for the three configurations were 0.319, 0.271, and 0.518 respectively. One of the things we intend on looking into more is how to increase the coverage. In terms of coverage the results were on average, 0.392, 0.251, and 0.170. The main goal of this work was to show that Spiking Neural Networks can get comparable performance to other prefetchers, in order to extend the range of Spiking Neural Network applicability, and to provide a different angle on Machine Learning based data prefetching possibilities.

Acknowledgements: Lin Jia and Ian Lavin

About the Author

James McMahon UNIVERSITY OF UTAH

## 19. **Reflection on**

### Undergraduate

### Research

### **Experience** -

### **Breanna Michele**

### Wong

Breanna Michele Wong (University of Utah)

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

Prior to knowing what research really was I had no idea how much it would mean to me now. I am so thankful for Dr. Kong and the members of the additive manufacturing lab that taught me the inner works of engineering research. My experience has taught me how to create my own hypothesis and follow through with experimentation. It has also taught me the importance of literature review and teamwork. Most importantly, it has taught me the virtue of patience as research can have its challenging bumps and not always go the way you expect it to go. I will be using this experience to help me as a future physician and hopefully get to conduct more research in the future.

About the Author

Breanna Wong UNIVERSITY OF UTAH

20. 3D Printing

Transient

### **Electronics for**

### Biomedical

### Implants

Breanna Michele Wong (University of Utah)

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

#### Reflection

In the midst of the coronavirus 2019 disease, I was in search of joining a research lab to improve my experiences for medical school. I applied to multiple research labs but I had difficulty getting interviews for one as during COVID-19 most labs were kicking out all of the undergraduate students to limit the amount of exposure. That is until Dr. Yong Lin Kong responded to my request to be a part of their lab. I still remember the details of our interview because I was so thankful for the opportunity that Dr. Kong gave me in the midst of a pandemic. I was honest and told him that I had no experience in the biomedical/mechanical engineering field (as I was a health society and policy major) but yet he told me that I would be able to trained and learn along the way. I am so thankful he took a chance on me and helped me find my passion for research.

Everything in research was new to me. Dr. Kong and I agreed to meet every week to connect on my progress and he would have a graduate student train/guide me along the way. I learned how to do research, specifically 3D printing research in the most hands on manner. I learned how to use a 3D printer, perform ink synthesis, and conduct my own experiments. Dr. Kong had me keep a journal that focused on describing my hypothesis-based experiments to help guide my research in a quantitative and qualitative way. We also had weekly team meetings with other members in the lab to share about what progress we've made in our research. This was also a great space to speak about challenges that we've faced throughout our research in order to get outside opinions.

I learned that research takes grit and patience. I gained to love the feeling of something finally going the way you had been working towards. I also enjoyed those 'eureka' moments of finding out why things weren't going the way you planned. I grew my knowledge on biodegradable transient electronics and found that research also has a focus on literature review. I gradually learned how to read scientific papers and how to connect them to creating a hypothesis for your own research.

I am also grateful for the Undergraduate Research Opportunity Program (UROP) that helped fund my passion for research. UROP allowed me to continue doing research without the limitations of cost while being an undergraduate college student. I also learned through UROP how to present my research. The program gave me the opportunity to present and engage with others on the details of my research.

Prior to knowing what research really was I had no idea how much it would mean to me now. I am so thankful for Dr. Kong and the members of the additive manufacturing lab that taught me the inner works of engineering research. My experience has taught me how to create my own hypothesis and follow through with experimentation. It has also taught me the importance of literature review and teamwork. Most importantly, it has taught me the virtue of patience as research can have its challenging bumps and not always go the way you expect it to go.

About the Author

Breanna Wong UNIVERSITY OF UTAH

**DEPARTMENT VI** 

# **College of Health**

# 21. Reflection on

## Undergraduate

### Research

## **Experience** - Sarah

## Haysley

### Sarah Haysley (Ohio State University)

Faculty Mentor: Skyler Jennings (Communication Sciences and Disorders, University of Utah)

This is easily one of the best academic and career experiences I have been a part of. I truly value the perspectives of research and interdisciplinary collaboration that I gained from the SPUR [Summer Program for Undergraduate Research] program. This program allowed me to participate in research more than the typical undergraduate program would allow. My mentor dedicated lots of time to answer my questions and develop research ideas with me. As a student who is not from the 180 Annie Isabel Fukushima (Editor in Chief)

University of Utah, this program was valuable because it allowed me to evaluate this university as a prospective graduate school in a hands-on experience.

About the Author

Sarah Haysley OHIO STATE UNIVERSITY

# 22. Assessment of

## an Auditory Reflect

## **That Facilitates**

### Listening in

### **Background Noise**

Sarah Haysley (Ohio State University)

Faculty Mentor: Skyler Jennings (Communication Sciences and Disorders, University of Utah)

### Background

Hearing in background noise is a significant listening challenge for individuals with hearing loss despite the use of hearing aids. Sound information is sent to the brain through neural responses from the afferent auditory system. These responses are modified by the efferent auditory system, which consists of neural feedback from the brain to the ear. Based on animal research, the medial olivocochlear reflex (MOCR), which forms part of the auditory efferent system, is hypothesized to facilitate listening in noisy backgrounds. Specifically, the MOCR limits the deleterious effect of background noise on auditory nerve responses, thereby, improving the neural signal-to-noise ratio. This improvement may be diminished with age and hearing loss and contribute to age- and hearing-loss related difficulties in understanding speech in noisy backgrounds. Clinical assessment of the MOCR is essential to understand the extent to which a patient's speech-in-noise difficulties may be explained by MOCR dysfunction. Yet, current assessments of the MOCR, based on otoacoustic emissions (OAEs), require individuals to have good hearing sensitivity. The purpose of this study is to develop an alternative assessment of MOCR function based on a measurement technique - the cochlear microphonic (CM) that produces reliable responses in individuals with hearing loss. The CM is an indirect measure of cochlear function and is sensitive to the effects of the MOCR on the response from the cochlea. When the MOCR is elicited by contralateral sound, the CM amplitude increases. As a first step toward developing a clinical test of the MOCR, we designed the following study to determine which sound frequencies result in the largest MOCR-induced increase in CM amplitude.

### **Research Design**

The effect of contralateral noise on the CM was measured for frequencies ranging from 100-6000 Hz using an electrode that rests on the tympanic membrane. Contralateral broadband noise (50 dB SPL) was presented to the left ear to elicit the MOCR while an ipsilateral test tone (probe) swept in frequency from 100 – 6000 Hz in the right ear at 90 dB

SPL. Six young adults (4F, 2M) completed two sessions in a randomized, counterbalanced order: one with a down-

sweeping and another with an up-sweeping probe. All subjects had normal hearing sensitivity, normal middle ear function, and no history of hearing loss or neurological problems.

### Results

Preliminary data revealed that CM amplitudes were consistently enhanced by 1 - 2 dB across a wide range of frequencies tested. We observed this enhancement for upward and downward frequency sweeps. Fine structure was observed in CM measurements with and without contralateral noise. The primary effect of the noise was to shift the CM fine structure towards higher frequencies.

### Conclusions

The results show that the CM amplitude increased in the presence of contralateral noise, consistent with eliciting the MOCR. This enhancement was for greatest frequencies between 250 and 2000 Hz suggesting that future clinical tests based on the CM should focus this frequency region. Similar effects of on contralateral noise have previously been reported in OAE assessments of the MOCR. This consistency among CM and OAE results support the CM as an appropriate alternative for measuring MOCR function. Yet, this study's design shows promise for assessing the MOCR in individuals with hearing loss. The next step in developing such tests is to extend this design to include older adults with normal hearing and hearing loss to test the hypothesis that a CM-based test is sensitive to the putative declines in MOCR function that result from aging and hearing loss.

About the Author

Sarah Haysley OHIO STATE UNIVERSITY

# 23. Reflection on

### Undergraduate

### Research

### **Experience** -

### Zachary Mallender

Zachary Charles Mallender (University of Utah)

Faculty Mentor: Christopher Depner (Health, Kinesiology, and Recreation, University of Utah)

I was able to assist in my lab through direct participant interaction, as well as have the agency to pose and answer my own scientific question throughout my research. Understanding the method by which research is conducted has made me a more thorough and effective reader of scientific works. Having medical research experience was an extremely rewarding part of my undergraduate career, and will hopefully be a significant help in achieving my future goals.

About the Author

Zachary Mallender UNIVERSITY OF UTAH

- 24. Assay of the
- **Dreem Device on**
- **Sleep Metrics and**
- an Exploration of
  - **Sleep Staging in** 
    - **Chronic Short**
  - **Sleepers During**

## **Time in Bed**

## Extension

Zachary Charles Mallender (University of Utah) Faculty Mentor: Charles Depner (Health, Nutrition, and Kinesiology, University of Utah)

Despite clear and plentiful research that sleeping less than seven hours per night has a wide array of health consequences, a large portion of American adults report sleeping less than seven hours per night and thus receive chronic insufficient sleep. Many studies exploring the consequences of insufficient sleep are restricted to small sample sizes and short recording significant cost to gold-standard times due to а polysomnography in terms of expense, time, and reliance on trained sleep technicians to prepare and monitor subjects. Additionally, most studies adopt a design of interventional sleep restriction on otherwise healthy sleepers, which excludes people who receive long term insufficient sleep over months to years. Here, we attempt to explore possible solutions to these issues through the use of a sleep extension study using the Dreem headband, a wireless dry electrode consumer electroencephalography (EEG) device, to measure overall sleep metrics and EEG data. When compared to wrist-mounted actigraphy (fig 1), the Dreem indicates little systemic skew for data over 75% quality (as assigned by Dreem), but reports significant random error with limits of agreement starting approximately 70 minutes off of actigraphy baseline. Exploration of sleep metrics in baseline insufficient sleep vs interventional sleep extension (table 1) revealed an increase in total sleep time; increase in all recorded sleep stages; and no significant changes in sleep onset latency, wakefulness after sleep onset, or sleep efficiency. Although several limitations of producing high quality data were identified, the Dreem headband shows promise as a home environment sleep research device. With an improvement in data quality the

Dreem, or another wireless consumer sleep device, has the potential to help advance the sleep field in ways that have traditionally proven inaccessible.



Figure 1: Bland-Altman plots showing comparative measure of total sleep time in minutes. Actiwatch data served as a reference device, which Dreem data was compared against. The red line represents calculated overall skew, and the solid grey lines represent the limits of agreement (LOA) for the two data sets. Dotted lines represent respective 95% CI. Panel A: all nights of data collected. Panel B: nights of greater than 85% quality. Panel C: nights of greater than 75% quality. Panel D: nights of less than 70% quality. Systemic skew underreporting sleep time for longer sleep episodes can be clearly seen in lower quality data, but is not present in green quality data. All data has roughly similar LOA, representing overall agreement of the Actiwatch and the Dreem headband, with a small increase in LOA for lower quality data. Data of 75% or higher virtually eliminates systemic skew.

| Sleep Stage | Baseline (minutes ± SEM) | Sleep extension<br>segment (minutes ±<br>SEM) | Sleep extension p value |
|-------------|--------------------------|---|-------------------------|
| N1          | $17.9 \pm 1.8$           | $22.9 \pm 1.7$                                | 0.003                   |
| N2          | $134.6 \pm 11.9$         | $192.7 \pm 10.6$                              | < 0.001                 |
| N3          | $71.9 \pm 6.0$           | 91.1 ± 4.4                                    | < 0.001                 |
| REM         | 82.5 ± 7.6               | $100.4 \pm 6.7$                               | 0.007                   |

### About the Author

Zachary Mallender UNIVERSITY OF UTAH
## 25. **Reflection on**

## Undergraduate

#### Research

## **Reflection - Keaton**

#### Rosquist

Keaton Rosquist (University of Utah)

Faculty Mentor: Anandh Velayutham (Nutrition and Integrative Physiology, University of Utah)

When I began my research for the summer, I had a lot of concerns and questions. I soon found that any concerns that I may have had previously were quickly resolved as I began to work with my lab group. Our research was extremely interesting as it had a lot that is clinically based. In our lab we utilized a lot of techniques that were taught to us

in classes. We utilized western blotting, PCR, and histology staining to view our results. I had the opportunity to design a research project. Study previous works relating to my own research concern and I learned how to implement a protocol to find data that would lead me to a conclusion. I have found a new love for the research field that I did not think was present before. I have seen the need for more people in the research field and what more we can learn. I want to become a medical doctor and research as I have further found out is an important way to understand what is wrong with the human body and what can be done to help the body. I know this experience will stay with me throughout my career in the medical field. I plan to implement my research skills and experiences in a way that will make me a better healthcare practitioner.

About the Author

Keaton Rosquist UNIVERSITY OF UTAH

## 26. The Influence

## of Prior Knowledge

## on the Learning of

#### **New Movements**

#### Across the Human

#### Lifespan

Jade Robinson (Johns Hopkins University); Bradley King (Health and Kinesiology, University of Utah); and Anke Van Roy (University of Utah)

Faculty Mentor: Bradley King (Health and Kinesiology, University of Utah)

The speed by which we acquire new knowledge critically depends on what we already know; new information is rapidly

acquired when it is compatible with previously learned and consolidated knowledge (i.e., an acquired schema). Relatively little is known about how this schema effect differs across the human lifespan. The current study aims to examine the schema effect across the human lifespan through the use of a motor sequence learning paradigm. Two hundred participants, divided into groups of children (7-12 years old), adolescents (13-17 years old), young adults (18-35 years old) and older adults (> 55 years old), will complete a bimanual Serial Reaction Time Task (SRTT) administered through an online data acquisition platform. Twenty-four hours after the acquisition of an initial motor sequence, participants will learn a new sequence that is either highly compatible or incompatible with the initial sequence. The effect of sequence compatibility will be examined across the different age groups, allowing us to assess the schema effect across the lifespan. The results of the study will contribute to the narrow selection of literature on the schema effect and motor learning; however, further investigation of how schemas influence motor learning is necessary to better understand how learning and motor movements inherently change throughout the lifespan and improve relevant clinical practices.

About the Authors

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Bradley King UNIVERSITY OF UTAH

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Anke Van Roy Van Roy UNIVERSITY OF UTAH

27. Blueberry

## Supplementation

#### Improves

## **High-Fat-Diet**

## Induced Gut

## Inflammation

Keaton Rosquist (University of Utah)

Faculty Mentor: Anandh Babu Pon Velayutham (Nutrition and Integrative Physiology, University of Utah)

#### Background

Obesity has currently been a growing health concern that has ranged across the world. Diet-induced obesity drives gut inflammation through the production of cytokines and alteration in gut microbiota. High fat diet (HFD) favors the conditions that lead to gut inflammation with an increased gut epithelial permeability that allows for higher chances of disorders occurring. Antibiotic gastrointestinal usages interrupt the gut symbiosis and further exacerbates HFDinduced complications. Evidence indicates HFD with antibiotics increases the risk of pre-inflammatory bowel (IBD). Blueberries contain bioactive flavonoid disease compounds called anthocyanins which possesses antioxidant and anti-inflammatory properties. In the present study, we assessed whether dietary blueberry improves HFD- and antibiotics-induced gut inflammation.

#### Methods

Male C57BL/6J mice (7 weeks old) were divided into three groups: (1) control mice consumed standard diet (C), (2) mice consumed HFD and treated with antibiotics in drinking water (HFA), (3) mice consumed blueberry supplemented HFD and treated with antibiotics (HFAB) for 12 weeks. Gut inflammation was assessed by measuring the mRNA expression of inflammatory markers (IL-1 $\beta$ , IL-6, iNOS and MCP-1) using qPCR. The total RNA was isolated from colon using RNeasy plus mini kit, cDNA was synthesized using RT-PCR kit, and the expression of inflammatory molecules was measured with qPCR by using SYBR green (Qiagen).

#### **Results & Discussion**

The mRNA expression of inflammatory markers IL-1 $\beta$ , iNOS and MCP-1 were significantly increased in HFA vs C mice. However, dietary supplementation of blueberry significantly reduced the expression of IL-1 $\beta$ , iNOS and MCP-1 indicating the beneficial effect of blueberries on gut. The mRNA expression of IL-6 was similar among the groups. Our ongoing studies are focused on identifying the molecular mechanisms involved in the protective effect of blueberries. Our study 198 Annie Isabel Fukushima (Editor in Chief)

suggests consumption of blueberry may be a potential dietary approach to improve gut health.

About the Author

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## 28. The Influence

## of Time-Restricted

#### Feeding on

## **Mesentric Arterial**

#### **Function in Older**

#### **Obese Mice**

Heba Sultan (University of Utah)

Faculty Mentor: J. David Symons (Nutrition & Integrative Physiology, University of Utah)

Endothelial dysfunction resulting from systemic inflammation and / or metabolic disruption precipitates age and obesity-associated vascular complications. Time-restricted feeding (TRF) prevents metabolic diseases in mice fed an obesogenic diet, but its effect on endothelial function in the context of obesity and / or aging is unknown. Here we tested

the hypothesis that TRF attenuates age and obesity-induced vascular dysfunction. Three-month old C57BL/6J male mice consumed high-fat high sucrose (HFHS) chow ad-libitum for 10-months. At 13-months of age, one group of mice continued HFHS feeding in an ad-libitum manner (ALF), whereas another group consumed HFHS chow between 0800-1700 only (i.e., TRF). Importantly, all mice were familiarized with and maintained on an altered light: dark cycle such that the dark phase occurred between 0800 and 1700. After 12-months of HFHS feeding, vasomotion was assessed in mesenteric arteries from 25-month-old ALF (106±4 um i.d.) and TRF (105±4 i.d.) mice (n=8 mice per group) using isometric tension procedures. Non-receptor mediated vasocontraction to potassium chloride (10-100 mM) was similar between groups. α-1 receptormediated vasocontraction to phenylephrine (PE; 10-8 - 10-5 M) was similar between groups. After precontraction with phenylephrine (PE) to ~ 70% of maximal PE-induced tension development, vasorelaxation to acetylcholine (ACh; 10-8 - 10-5 M) was greater (p<0.05) in TRF vs. ALF mice, whereas responses to sodium nitroprusside (SNP; 10-9 - 10-4 M) were similar between groups. These preliminary findings indicate TRF preserves mesenteric artery endothelial function in obese, aged mice. TRF might be an efficacious lifestyle modification strategy to lessen obesity- associated disruptions to vascular health in the context of aging.

About the Author

Heba Sultan UNIVERSITY OF UTAH

**DEPARTMENT VII** 

# College of Humanities

29. Reflection on

Undergraduate

Research

**Experience** -

#### Gabrielle

#### Desjardins

Gabrielle Desjardins (University of Utah)

Faculty Mentor: Catherine Goodman (Writing & Rhetoric Studies, University of Utah)

The research I have taken part in as an undergraduate is independent of the work I am currently submitting, but it is still relevant. I currently work in Dr. Katharine Diehl's lab as an undergraduate researcher. The title of the project is "Development of fluorescent biosensors for acyl-CoAs." I was interested because the title had something I knew (acyl-CoAs) and combined it with something I was vaguely familiar with (biosensors). I knew acyl-CoAs were metabolic intermediates, and I had heard about fluorescent proteins in my general biology course and thought they were interesting. When I met with Professor Diehl to discuss the advertised project, I was fascinated. I did well in my biochemistry courses, but as she described the goal of the project, I realized that there were so many things I still did not know. As I have participated in the Diehl lab, I have learned more about the numerous complexities of biochemistry.

About the Author

Gabrielle Desjardins UNIVERSITY OF UTAH

## 30. An Activity to

#### **Increase Interest in**

## STEM Among

## Historically

#### Underrepresented

#### Students

Gabrielle Desjardins (University of Utah)

Faculty Mentor: Catherine Goodman (Writing and Rhetoric, University of Utah)

#### Abstract

In today's society, occupational opportunities relating to science, technology, engineering, arts, and mathematics (STEM) are increasing. Unfortunately, there is an unequal representation of minorities in STEM-related occupations. In an article written by Jennifer Gunn from Concordia University, she explains that the unequal distribution of people of color in the workplace begins in the grade-school setting. Children of color are less likely to be exposed to STEM in their classrooms, ultimately contributing to their disinterest in pursuing STEMrelated careers in the future (Gunn). This lab report outlines an experimental procedure to transform a mixture of milk, vanilla extract, and sugar with rock salt and ice into ice cream. The students will use observational techniques to see that lowering the temperature will cause the mixture to change from a liquid to a solid state. The experiment explores the transition and cause of transition between the three states of matter. The purpose of this lab report is to provide a low-cost activity to instructors and to make STEM more accessible to minority students, so there is an equal opportunity for everyone.

#### Introduction

A significant number of occupational opportunities originate from the fields of science, technology, engineering, and mathematics (STEM). Unfortunately, there is a gap in the number of minorities in the STEM field. According to Jennifer Gunn from Concordia University, about 70% of the STEM occupation population are white men or women. She attributes this disparity to how minority children are underexposed to STEM in their grade-school years (Gunn). One possibility for underexposure is inadequate funding from the State Board of Education. Without proper funding from the state to perform STEM activities, the teacher would be financially responsible for the entire experiment, and they may not have the resources either. A second reason for underexposure is that the instructors do not have adequate training in STEM. This lab report proposes a way to counteract these challenges by providing explicit instructions for a low-cost experiment.

Consequently, instructors can expose their students to STEM in an engaging, fun, and inexpensive way.

An essential concept to grade-school education is the three states of matter. An experiment to turn this concept into an interactive learning experience is to make ice cream using milk, sugar, vanilla extract, ice, rock salt, and plastic bags. The first objective is to educate students on the three states of matter. An applicable example is placing ice (a solid) into a pot on a heated plate. The ice will melt into water (a liquid). Continuing to apply heat will cause the water to boil and emit steam (a gas). The second objective is to hypothesize the effect of reducing the milk's temperature, which will allow the opportunity for post-experimental discussion as a class. The final objective is to establish a community in the classroom. The students will work as a team throughout the experiment, which will teach them to work together–an essential skill in STEM.

#### Method

The recipe for the experiment was adapted from the ice cream recipe by Kidzworld and required the following materials: rock salt, skim milk, soy milk, lactose-free skim milk, half and half, white sugar, vanilla extract, ice, a thermometer, measuring cups, 1-gallon and 1-quart plastic bags, spoons, and a small bowl. The instructor prepared the ice cream mixture before the classroom time by combining one cup of milk, two tablespoons of sugar, and one-quarter teaspoon of vanilla extract to a 1-quart plastic bag. The 1-quart plastic bag was sealed airtight, then placed into a second 1-quart plastic bag and sealed.

In the classroom, the experiment followed a short lesson about the states of matter. The lesson was completed by showing a video titled "Bill Nye the Science Guy Phases of Matter," but could have been completed by a verbal lecture from the instructor. After the lesson, the students washed their hands and cleaned their desks. Next, the students were paired up and given a worksheet (Appendix A) to be completed throughout the experiment. Finally, the instructor guided the students with the following procedure:

- Each pair of students received a spoon, bowl, and a bag containing the prepared mixture. The bag containing the mixture, one scoop of rock salt, and two cups of ice was placed into a 1-gallon plastic bag. The bag was closed tight to avoid any spills.
- 2. A thermometer was used to measure the initial temperature of the mixture (Figure 1) and recorded on the worksheet from Appendix A. At this point, the teacher reminded students to record the temperature and their observations every three minutes on their worksheets.
- The bag was shaken for three minutes, then exchanged by partners. After each three-minute interval, the temperature was measured and recorded.



Figure 1: How to Measure the Temperature of the Ice Cream Mixture

After four intervals (twelve minutes) passed, the ice cream was frozen and ready for consumption. The students scooped equal portions of the resulting ice cream into two bowls by using the spoon they received at the beginning of the experiment. Every student was involved in the clean-up process. Once everything was clean, the instructor completed the lecture by allowing the students to discuss their results, observations, and examples of the three states of matter.

#### Results

The results from the experiment were used to create the figures in Appendix B. Figure 2 shows a tabular representation of the recorded temperature at each time interval, and Figure 3 shows a graphical representation of the data for visual comparison. All trials were successfully converter from liquid to solid, with the exception of the soymilk trial. A picture of the final product for the four trials is displayed in Appendix C.

#### Discussion

The mixture was expected to freeze after ten minutes of

shaking; however, at the end of the first trial, the soymilk trial, the mixture was still partially in liquid form. The cause of this was attributed to the ice melting after about six minutes. In an attempt to produce more efficient results, two extra scoops of ice were added to the plastic bag at five minutes on the second trial. Additionally, two minutes were added to the overall time spent shaking the bag. This modification achieved the desired results for the remaining trials.

Another unexpected variation was found in the initial temperatures. The source of this variation coincides with the amount of time each milk was outside of the refrigerator. The soy milk was left at room temperature for the most extended period and had an initial temperature of 7.6 °C. The elevated temperature may have contributed to the final product not solidifying completely. The mixture should be kept either in a cooler or a refrigerator until it is given to the children to avoid higher initial temperatures in the future. Then, the mixture should be immediately added to the gallon-size plastic bag with the rock salt and ice. These modifications provided the most desirable outcome.

#### Conclusion

The experiment demonstrates the states of matter by freezing a liquid mixture of sugar, vanilla extract, and milk using rock salt and ice. Throughout the experiment, the temperatures of each mixture are recorded in three-minute intervals. From this, students learn that reducing the temperature (energy) decreases the motion of the molecules. Although the mixture from the soymilk trial did not freeze completely, the final procedure was adapted to limit the possibility of undesirable outcomes. The purpose of the experiment is to provide an accessible way for minority students to experience STEM. The cost of the ice cream experiment is \$16.50 for a class of thirty students. This lab report also provides an explicit, yet simple guideline on how to perform the states of matter experiment. With these tools, children from underrepresented communities are able to explore the field of STEM effectively.

Appendix A: Student Worksheet Name 1: Name 2:

Complete the drawing:

1. Write the state of matter in the line above the picture.

2. Draw the what the molecules look like in each state of matter in the boxes below. 3. Near the arrows, write the name of the change to get to each state of matter.



Now you are ready to begin your experiment:

A hypothesis is what you think will happen in the experiment. 1. What is your hypothesis for this experiment?

2. Record the temperature of your mixture 3. Record your visual observations here: every three minutes in the table below:

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2. Record the temperature of your mixture every three minutes in the table below:

| Time Shaking | Temperature (°C) |
|--------------|------------------|
| 0            |                  |
| 3            |                  |
| 6            |                  |
| 9            |                  |
| 12           |                  |

3. Record your visual observations here:



#### Appendix B: Experimental Data

| Time Spent<br>Shaking with<br>Ice and Rock<br>Salt (in | Temperature of | Temperature of | Temperature of<br>Lactose Free | Temperature of |
|--|----------------|----------------|--------------------------------|----------------|
| Minutes)   | Skim Milk      | Soymilk        | Milk                           | Half and Half  |
| 0  | 1.6 °C         | 7.2 °C         | 3.4 °C                         | 5.2 °C         |
| 3  | 0.1 °C         | -1.1 °C        | 0.4 °C                         | 0 °C           |
| 6  | -0.1 °C        | -2.6 °C        | -2.4 °C                        | -2.8 °C        |
| 9  | -0.9 °C        | -2.8 °C        | -3.2 °C                        | -3 °C          |
| 12   | -4.6 °C        | -3 °C          | -3.8 °C                        | -3.2 °C        |

## Figure 2: Table of Mixture Temperature Over a Twelve-Minute Shaking Period



Time Spent Shaking Bag (minutes)

Figure 3: Graph of Mixture Temperature Over a Twelve-Minute Shaking Period

Appendix C: Final Product Appearance

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About the Author

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**Education on** 

Objectification

Theory and Why it

is More Beneficial

for Women's Issues

#### than the Body

#### **Positivity**

#### Movement

Lauren Lloyd (University of Utah)

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#### ABSTRACT

The Sexual Objectification Theory proposed by Barbara Fredrickson and Tomi-Ann Roberts postulates that the sexual objectification of women can lead to depression, eating disorders, and sexual dysfunction. Further research on this theory has shown that objectification is widespread and has several consequences such as diminished mental health in women and increased rates of violence against women. This phenomenon and its effects have worsened with the advent of social media. On social media platforms, women are frequently exposed to sexually objectifying content and unattainable beauty standards. The body positivity movement combats the destructive messages in the media by empowering women through realistic representations of women's bodies. While the body positivity movement has made great progress in the realm of objectification, the movement is divisive and limited in its capabilities. To reduce the rampant objectification in society, we must formally educate future generations on objectification and its ramifications. Therefore, I propose a lesson plan for the Utah Core Standards of three, 45-minute class periods. The curriculum contains two PowerPoint presentations, two videos, two images, one article, one activity, one handout, and one Socratic seminar.

#### Introduction

I was objectified for the first time when I was 12 years old. The boys in my grade decided to craft the "perfect girl's body" using different physical features from the girls in our grade. Word got around about whose body parts they selected. When I found out which part of my body was chosen for this "perfect girl," I was mortified. I look back on this experience and pity that poor 12-yearold girl, who had just realized for the first time in her short life that she had no control over her own body. Instead, her body was now controlled by the boys who viewed her as an object first, and a person second. This experience opened the floodgates for a torrent of sexually objectifying experiences that I would face during my adolescence.

After I hit puberty, my mother would become enraged when we walked in public together and she saw the way adult men ogled me. School administrators reprimanded me for wearing clothing permitted by the dress code because my chest developed earlier than the other girls in my grade. College-aged men catcalled at me anytime I walked by the local university. I was given the nickname "thick thighs" in high school. Unfortunately, my experience is not unique. Sexually objectifying experiences function as a disgusting and upsetting rite of passage for young girls into womanhood.

Sexually objectifying experiences occur when women are reduced to their bodies, or a collection of their body parts, for the gratification of other people.1 These experiences can occur within women's daily interactions and also online when they come across sexually objectifying media. Sexually objectifying media emphasizes women's bodies over anything else, as if their bodies alone are able to represent them as a whole.2 In the past decade, social media has become a predator, and its prey are young, vulnerable girls who have no defense against objectification, body shaming, and sexualization. On these platforms such as Instagram and TikTok, they are subjected to extremely limited representations of what women are supposed to look like. Advertisements follow suit on their timelines and convince girls that they are worth nothing unless they are free of acne, body hair, fat, cellulite, stretch marks, or any imperfection. Then, they are bombarded with videos and images of women in minimal clothing who are showcased for male approval. Just by existing in the world as a young woman, whether she is at school or work, scrolling on social media, or minding her own business in public, women witness an obscene amount of objectification.

As I got older, I began to notice the toll that objectification was having on myself and the women around me. It broke my heart to see my best friends scrutinize every aspect of their appearance and degrade themselves. I empathized with them when they broke down sobbing over a picture of themselves that they didn't like. I noticed patterns of restricted eating with the women in my life, especially for those who had lived through the '90s. It was normal to hear comments such as "good thing I worked out today" or "guess I won't be eating tomorrow" anytime I sat down for a large meal with women. These women whom I loved and valued for their kindness, their intelligence, and their humanity hated themselves just because they didn't look like the fictitious images of women who were praised in the media. These examples represent casual and normalized consequences of objectification. In this thesis, I will highlight the more dangerous side effects of objectification including eating disorders, depression, sexual dysfunction, and violence against women.

I spent all of my high school years envying my friends' physiques, all the while they were wishing that they looked like somebody else. Women are constantly comparing themselves to other women. It doesn't matter what a woman looks like; as long as objectification is ubiquitous in society, she will never be content with her appearance. However, my and many other women's self-image improved tremendously once we were exposed to the body positivity movement. The movement stems from the fat rights movement which was founded in the 1970s. Once fat rights activists took to Tumblr and Instagram during the early 2000s, it was rebranded as the body positivity movement by the new generation.3 Activists within the movement highlight the authentic version of themselves and give women tools to build their confidence. Seeing a variety of body types positively represented in the media helps women accept themselves as they are.

I became enthralled with the body positivity movement, and I wanted to share it with other women who struggled with their body image. Therefore, I began this thesis in my sophomore year of college. It started out as an enterprise story for my journalism class about the body positivity movement. I interviewed three accomplished women who shared their insights and experiences of objectification with me. I wrote this piece, and it was published on UNewsWriting.org. Yet, when it was finished, I felt like the work had just begun. I had so much more to say and learn about the objectification of women. So, the following year, I began my research for this thesis. I pored over a plethora of academic papers and carefully parsed school curriculums. At times, it felt heavy. I was overwhelmed by the stories of all the women who were suffering. This is when I realized why this work is so important. If I wanted my mother, my grandmother, my friends, and all of the inspiring women I know to value themselves the way that I valued them, something needed to change.

The body positivity movement has several benefits for women. such increasing as representation of body types, improving women's body image, and creating an empowering online community. These changes are not enough. Fortunately, there is an abundance of resources to help women unlearn the self-hatred that objectification has instilled in them. However, it is an arduous process to unlearn the values that have been taught to you since you were a child. Women have to suffer the effects of objectification, and then do the work to dismantle it as well. Furthermore, there are so many differing opinions and factions within the movement that its effectiveness has been diminished. Critics of the movement discuss how it perpetuates an emphasis on physical appearance, and it largely benefits white women. Some believe that body positivity should be for everyone. Others want to emphasize that body positivity is different for plus-sized women and women of color because our culture places the most value in white, thin bodies.4 When women are further pitted against other women, progress is derailed.

I propose that the solution to this issue is to educate boys about the harm of objectification. If men and women are equally informed on the effects of objectification, there is a higher chance of altering objectifying social norms. Women are perpetually surrounded by objectification, so they can easily comprehend when it is occurring and how it will detrimentally impact the victim. Men do not have the same perspective on objectification because they are not as frequently the subjects of it. Objectification can no longer be just a women's issue. Women can work on their confidence and self-esteem for their entire lives, but the problem will not improve for the following generations if the issue is not attacked head-on.

To confront the problem of objectification directly, I designed a school curriculum for 7thand 8th-grade health students in Utah. I posit that teaching youth about objectification will make objectifying attitudes less normalized in society, which will improve girls' mental health and reduce rates of violence against women. In this curriculum, students will learn what objectification is, the negative impacts of it, and how they can be a part of the solution. I chose to include this lesson plan for 7th- and 8th-grade students because it naturally fits into the content of the Utah Core Standards and would be simple for teachers to implement. This is also a crucial time for teenagers' development. I was objectified for the first time when I was 12, when I was in the 7th grade. Maybe if those boys had studied the harmful effects of objectification, then that 12-year-old girl would not have learned that her worth as an individual lies in her appearance.

#### Literature Review: Objectification Theory

The Sexual Objectification Theory was first proposed in 1997 by Barbara Fredrickson and Tomi-Ann Roberts. Their theory posits that women are socialized to internalize objectification and treat themselves as objects.5 This is referred to as selfobjectification, and it has a detrimental impact on women's mental health.6 Fredrickson and Roberts determined two routes in which women arrive at these serious mental health problems. First, when women are sexually victimized through rape, incest, assault, or sexual harassment, there is a direct pathway to depression, eating disorders, and sexual dysfunction.7 Second, when women internalize the proliferation of objectification in society, there are physiological consequences that manifest into these larger mental health issues.8 example, self-objectification results For in appearance anxiety and body monitoring, which leads to depression and disordered eating.9 Selfobjectification also leads to diminished internal awareness, body shame, and general anxiety, all of which may result in sexual dysfunction.10 The connection between self-objectification and diminished mental health is perpetuated by visual media, such as films, advertisements, television, music videos, and women's magazines that contain sexualized images of women, and interpersonal encounters.11 This is why the pervasive nature of social media so damaging for is women. Fredrickson and Roberts assert that this sexualization of women in the mass media is so ubiquitous that most, if not all women in American society are impacted by it.12 This is the seminal study in this area of research, and scholars are still expanding on it to this day.

#### **Mental Health Impacts**

A 2019 study has explored how this frequent exposure to sexual objectification in daily life negatively impacts women's everyday emotions. The researchers determined that objectifying events, such as catcalling or car honking, sexual remarks made about the body, nonconsensual touching or fondling, and sexually degrading actions still induce self- objectification in women even if not experienced firsthand.13 However, the tendency to self- objectify was strongest when were personally targeted by women the objectifying behavior.14 Most importantly, the researchers' evidence suggests that experiencing and witnessing sexual objectification in daily life increases body monitoring in women, which, in turn, intensifies women's experiences of negative and self-conscious emotions.15 Yet. the researchers identified that sexually objectifying events had a short-term psychological impact on women.16 They recognized that these short-term impacts may substantiate the claim from objectification theory that women develop coping skills to minimize the detrimental psychological effects of sexual objectification, which creates resiliency against them.17

In addition, research conducted by Dawn Szymanski and Stacy Henning contended that the psychological consequences of body monitoring—such as reduced concentration, greater body shame, and greater appearance anxiety—lead to depression.18 This research reaffirms the findings of objectification theory and provides further evidence that it is unlikely that the consequences of self-objectification are insignificant or short-term.

Sexual objectification is not an inconsequential The experiences that diminish phenomenon. objects inflict and compound women to psychological distress, especially when the "ideal body" perpetuated by the male gaze is unattainable for most women. In Brit Harper and Marika Tiggemann's study on the effect that the thin body standard has on women's well-being, they hypothesized that participants who viewed images thin idealized woman would featuring a demonstrate higher levels of self-objectification, appearance anxiety, negative mood, and body dissatisfaction than participants who viewed the control images.19 The results supported their hypothesis and revealed that even subtle stimuli can induce self-objectification in young women.20 Therefore, it is likely that women self-objectify several times a day.21 They suggest that recurring exposure to media featuring a thin idealized woman could place women at risk of developing depression or eating disorders.22

This concept was corroborated by Tracy L. Tylka and Melanie S. Hill's research on objectification theory and eating disorders. Their study addresses how objectifying messages, such as the societal pressure to be thin, could encourage women to focus more on their appearance and compare their bodies to other women's. They suggest that this

body monitoring and comparison leads to disordered eating.23 When a person negatively comments on a woman's weight or appearance, it indicates to her that she does not meet the thinideal beauty standard. Therefore, for this study, objectification is more broadly referred to as appearance feedback.24 When a woman receives negative appearance feedback, it signals to her that the way to obtain respect from others is to become skinnier.25 Tylka and Hill propose a model that explores how appearance feedback correlates to body surveillance, body comparison, body shame, and low self-esteem, and disordered eating (Fig. 1).26 To investigate these pathways, they had 274 women from a Midwestern U.S. college complete a questionnaire that measured the variables in their model. The data supported each correlation that Tylka and Hill put forward. Furthermore, their research demonstrated that women who frequently monitor their bodies and compare themselves to other women report the highest disordered eating.27



Fig. 1 Tylka and Hill's proposed model that predicts how appearance feedback leads to body surveillance, body comparison, low self-esteem, and disordered eating.

Most of the existing research that has backed the connection between sexual objectification and disordered eating has been conducted with college-aged women.28 Tracy L. Tylka performed another study with Casey L. Augustus-Horvath to explore if this connection was still prevalent for older women. In line with objectification theory, their model proposed that experiencing or witnessing sexual objectification causes a woman to self-objectify. Tylka and Augustus-Horvath posit that self-objectification leads to body shame, poor introspective awareness, and disordered eating (Fig.2).29 In this study, women were placed into two age groups: 18-24 years old and 25-68 years old. The women filled out two survey forms that measured the variables their model projected.30 The data supported their model, upholding that sexual objectification does lead to body shame, poor introspective awareness, and disordered eating for women above 25 years old.31 However, the interactions among variables within the model differed for younger and older women. They concluded that while older women reported lower levels of perceived sexual objectification and body surveillance than younger women, they reported similar levels of body shame, poor introspective awareness, and disordered eating.32 The negative effects of sexual objectification on women's eating behaviors are not unique to women who meet youthful beauty standards present in the media.33


Fig. 2 Model that illustrates how self-objectification leads to body shame, poor introspective awareness, and disordered eating, as proposed by Tylka and Augustus-Horvath. Dashed lines were not explored in this study.

It is important to acknowledge how sexual objectification affects women with multiple different identities. Fredrickson and Roberts assert that sexual objectification may impact women of color, women at lower socio-economic levels, and lesbians differently because of the additional oppressions they face in addition to being a woman.34 Many of the studies that extend objectification theory only include cisgender women in their data collection, since the theory was originally intended to explain how widespread sexual objectification affects cisgender women's well-being.35 Future research should explore how sexual objectification impacts transgender and non-binary individuals. Research has been conducted to investigate how the effects of sexual objectification differ for lesbian women. Holly B. Kozee and Tracy L. Tylka assessed whether

the variables of objectification theory that predict disordered eating, such as body shame, body surveillance, and lower internal awareness, apply to a sample of lesbian women.36 Lesbian and heterosexual women were split into two groups and were asked to fill out surveys that would quantify facets of objectification theory.37 This study revealed that the data from the heterosexual participants matched the model of objectification theory while data from the lesbian participants did not. The researchers found that lesbian participants had lower levels of disordered eating and greater levels of body surveillance than the heterosexual participants.38 This finding is consistent with objectification theory in that it supports that lesbian women experience the effects of sexual objectification differently than heterosexual women.

Research has also shown that there is а significant difference between how women of color and white women experience the consequences of objectification. As previously sexual noted, objectification theory postulates that rape, sexual assault, and sexual harassment lead directly to eating disorders, depression, and sexual dysfunction. In a 2015 study, researchers found that Black women reported more sexually objectifying experiences and fear of crime than white women.39 This may be attributed to the lack of bodily autonomy that Black women have historically

experienced. Researchers also cite overly sexual stereotypes of Black women in culture and media.40 Consequently, as women of color typically experience more sexual objectification due to racism and stereotypes, the mental health impacts are likely to be greater for them.

#### Violence Against Women

Sexual violence is a form of sexual objectification because a woman is treated as a sexual object that is separated from her humanity.41 To the perpetrator, her body and its sexual functions become more important than her ability to give consent.42 To investigate the connection between violence against women and objectification, Meghan Davidson and Sarah I. Gervais conducted research that explored how sexual violence and intimate partner violence relate to self- objectification, body surveillance, and body shame. Nearly 500 collegeaged women took a survey with questions that measured categories and frequency of sexual victimization, psychological and physical abusive behaviors, self-objectification, body surveillance, and body shame.43 The scholars' research extends objectification theory by contending that objectifying experiences do not have to be sexual. They suggest inherently that the dehumanizing nature of violence within a romantic relationship could predict the effects of objectification.44 Their data indicated a positive correlation between intimate partner violence and

self-objectification, body surveillance, and shame. However, regarding sexual violence, there was only a positive correlation between body surveillance and body shame.45 These findings could suggest that the relation between objectification and sexual violence is reversed: objectification leads to sexual violence.

Sarah Eagan and Sarah J. Gervais researched this missing link between sexual objectification and sexual violence. They assert that the ubiquity of objectifying attitudes, especially among men in power, encourages sexual violence against women.46 As their research shows, viewing women as objects is a likely first step toward committing sexual assault or harassment against them.47 There are two ways in which sexual objectification can lead to sexual violence. First, men who objectify women will be more prone to aggression against women. Second, these men can change norms about what is regarded as acceptable appropriate conduct from and toward men women.48 In addition, in our internet-obsessed society, the line between reality and the media is blurred, which contributes to the normalization of objectification.49 The cultural pervasiveness these factors create has direct implications for sexual assault.50 In their lab, the researchers found objectification to be a catalyst for alcohol- related sexual assault.51

To further the conversation about the harmful

effects of objectification in the media, Silvia Galdi and Francesca Guizzo put forward the Media-Induced Sexual Harassment framework.52 Τt illustrates three mechanisms through which sexually objectifying media lead to sexual the dehumanization of women. harassment: decreased empathy with women, and a shift in gender norms.53 The framework then addresses the effects that sexually objectifying media has on the perpetrator, victim, and bystander of sexual harassment.54 In their synthesis of preexisting evidence, they determined that all three of these mechanisms were responsible for the relationship between objectifying media and the perpetrator's increased likelihood to sexually harass women, as well as a delay in bystander intervention.55 Furthermore, dehumanization and a shift in gender correlated to the victim's increased norms for sexual harassment.56 tolerance Sexual objectification of women is commonplace in nearly of media, including television, forms all advertisements, music videos, and the internet.57 This framework demonstrates why this is so concerning for women, as it proposes that sexually objectifying media condones sexual harassment and therefore makes it more likely to occur.58

A 2021 study investigated if a video campaign against sexual objectification would reduce male sexual harassment against women. In this experiment, men were shown either a media campaign against female sexual objectification, video clips of a nature documentary, or video clips of sexually objectified women.59 After this, they were given three tasks that would test for three variables: sexually-harassing behavior, hostile sexism, and sexual coercion.60 According to Silvia Galdi and Francesca Guizzo's data, the media campaign produced the lowest levels of the variables and the sexually objectifying video clips produced the highest.61 The empirical data of this study also suggest that these results are not negligible.62 This study reveals how sensitizing media can be employed to counteract the omnipresence of sexual objectification in the media.63 The timeliness of this study also exemplifies the pressing nature of this conclusion. Intervention is possible at this moment. However, if sexual objectification becomes more pervasive than it is now, sensitizing campaigns may become futile.

#### The Body Positivity Movement

To reduce the harmful messages about women's appearance in the media, and the negative impacts they have, the body positivity movement was born. Users on social media began to celebrate realistic and natural representations of women's bodies. Images depicting women of different shapes, colors, and sizes, with stretch marks, soft stomachs, and other perceived "flaws" challenged the dominant beauty standards.64 The body positivity community was a safe space for women until corporations and influencers took over the movement. A 2021 study looked at how the commodification and reappropriation of the body positivity movement has detrimentally impacted the movement's purpose.65 Kyla N. Brathwaite and

David C. DeAndrea explored how social media users' ability to recognize when a company or individual uses the body positivity movement to promote their product or platform would impact their perception of the movement itself.66 They asked 851 women to view 10 Instagram posts that were each assigned to one of four conditions: posts with a body-positive message, posts with a bodypositive message and self-promotion, corporatesponsored posts not about appearance, and corporate-sponsored posts about appearance. After viewing these images, the women would then complete a questionnaire.67 The results from the questionnaire illustrated that the more participants recognized the presence of self-promotion or corporate advertising, the less morally appropriate and less effective at promoting body-positivity they viewed the post. However, these conditions less negatively impacted the viewer' perception if the woman in the post did not adhere to Western beauty ideals.68 This study reveals how bodypositive posts from women who conform to ideal body standards and/or contain self-promotion or advertising appear as disingenuous, and therefore less successful at encouraging body appreciation.69

Helana Darwin and Amara Miller's research also reveals the divisions and shortcomings within the body positivity movement. In their study, they analyzed 50 prominent blog posts from 2014 and 2016 to determine how feminist online spaces are

shaped by unequal power dynamics.70 Their investigation found that there are four different frames within the body positivity movement: Mainstream Body Positivity, Fat Positivity = Body Positivity, Radical Body Positivity, and Body Neutrality. The key differences between these frameworks are how advocates believe privilege should be incorporated within the movement, and whether body positivity should focus on individual psychological distress or societal size/race discrimination.71 Mainstream Body Positivity was the most popular frame among the blog posts, and it emphasizes self-love as the solution to the objectification of women. It asserts that the empowerment that comes from feeling and looking beautiful is the solution to body image issues. Many activists are critical of this frame as it reinforces objectification by centering a woman's worth in her appearance.72 Fat Positivity = Body Positivity was the second most prominent ideology. It advocates for recentering the movement on the systemic discrimination that fat experience. women Individuals disagree with this frame because they find it exclusionary of a multitude of women.73 Radical Body Positivity also agrees that the body positivity movement should focus on systemic oppression, rather than individual concerns about their bodies. It believes that addressing the root causes of size/race oppression will benefit all women. This was the least popular frame among

the blog posts.74 Lastly, the Body Neutrality frame accepts anyone who struggles with body image issues, regardless of their social privilege. It encourages women to feel neutral about their appearance, rather than aiming to always feel positive about their bodies.75 These findings elucidate how the body positivity movement is not just nuanced; it is incredibly divisive.76 Because the movement has become polarized and saturated with companies and influencers, the body positivity movement may not be the best solution to deal with the numerous detrimental effects that objectification theory proposes.

Lindsay Kite and Lexie Kite are identical twin sisters who have done groundbreaking work within the body positivity movement with their book More Than a Body: Your Body is an Instrument, Not an Ornament and their nonprofit organization Beauty Redefined. They propose that the solution to the objectification of women is body image resilience.77 Their research employs the idea of resilience as a method to improve body image. They define resilience as "being disrupted by change, opportunities, adversity, stressors or challenges and, after some disorder, accessing personal gifts and strengths to grow stronger through the disruption."78 The path to body resilience ensues after women experience body image disruptions, such as idealized images in the media or selfcomparison.79 They suggest that the first step to body resilience is called "sinking to shame." This occurs when women experience intense shame that can manifest into harmful behaviors such as disordered eating, self-harm, or drug abuse.80 The

second step is "clinging to your comfort zone" in which women try to change their appearance through makeup, clothing, or cosmetic procedures.81 The third and final step is "rising with resilience." This takes place when a woman acknowledges these body image disruptions, and chooses to rise above them, rather than feeling shameful or trying to change how her body looks.82 Their work is based on the idea that the objectification of women cannot be dismantled because it is so deeply ingrained in society.83

However, I suggest that dismantling is the next step of the body positivity movement. Women are the victims of objectification. They should not be solely responsible for the hard emotional labor of unlearning their self-objectification while society gets to continue to dehumanize them. In a 2010 study, researchers found that women were significantly more likely than men to detect the negative emotions caused by self-objectification.84 They hypothesize that this is due to men's limited experience with objectifying experiences.85 I argue that educating men and women equally on the dangers of objectification is the most productive way to change the objectifying attitudes present in society.

#### Introduction to the Curriculum

In this section I propose a curriculum about the objectification of women for students in 7th- and 8th-grade health. The curriculum is built on the information presented in this

literature review. Its purpose is to dismantle the objectification of women through the education of future generations. I designed this curriculum to match the methods of instruction that are included in the Utah Core Standards.86 It will consist of two PowerPoint presentations, two videos, two images, one article, one activity, one handout, and one Socratic seminar. In Utah's Core Standards for education, students complete Health 1 in 7th or 8th grade and Health 2 sometime during 9th-12th grade. There are six strands in Health 1 and 2: Health Foundations and Protective Factors of Healthy Self; Mental and Emotional Health; Safety and Disease Prevention; Substance Abuse Prevention; Nutrition; and Human Development. Each strand is comprised of related topics that mirror each other in the different grade levels. However, the content within the topics changes as the students progress through their education. For example, in the Mental and Emotional Health strand in Health 1, students learn about the risk factors for the development of mental health disorders, and in the Mental and Emotional Health strand in Health 2, students learn how media use can impact their mental health.87

A few strands include topics that pertain to the objectification of women. It would be easy to expand on any one of these areas to include a more in-depth lesson on objectification. In the Nutrition strand in Health 1, students are taught about positive body image. They learn how media and advertisements negatively alter their self-worth. They also learn how to value themselves as a whole person, rather than just as an image. In Health 2, this is mirrored by a lesson plan about eating disorders. In addition, in the Safety and Disease Prevention strand in Health 2, students watch a Dove campaign that shows how women's images can be heavily

86 "Utah Core Standards – UEN," accessed March 18, 2022, https://www.uen.org/core/. 87 "Utah Core Standards – UEN." distorted. However, this video is utilized to teach about the harmful effects of pornography, not about the harmful effects of objectification. Lastly, in the Safety and Disease Prevention strand in Health 1, students learn about the effects of media and technology on their health. In this lesson plan, students are taught about the differences in how men and women are used in advertising. This lesson plan could function as the perfect segue to teach students specifically about the objectification of women.88

If students learn to identify objectification and understand the harm of it, they will react negatively when they see it advertising and employed the media. Then. the in objectification of women will no longer be profitable for corporations. In addition, objectification will become less prominent on social media if students understand the dangers of objectifying content. To support this, research suggests that encouraging individuals to consider the negative impacts of objectification could lessen the prevalence of sexual objectification in our culture.89

The implementation of this curriculum could potentially lower rates of sexual harassment against women. As aforementioned, researchers found that men were less likely to engage in sexual harassment and sexual coercion after being introduced to the video campaign "Women, Not Objects." This campaign produced results in mitigating sexual harassment against women, thus it will be included in the curriculum. In the study, researchers showed participants "We Are Not Objects" and "#IStandUp Against the Harm Caused By Objectification of Women in Advertising." Since these videos feature mature content, students will watch "What Our Kids See" in the curriculum instead. This video reveals how young children internalize objectifying images and how it impacts their perceptions of themselves and others. This is another video from the same campaign, and it ensures the content is appropriate to show in schools.

### **Background on Objectification for Teachers**

This section is a summary of the information presented in this thesis to prepare teachers to lead the lessons on objectification. The definition of objectification for this lesson plan is "when a person is seen or treated as an object." This curriculum focuses specifically on the sexual objectification of women. Sexual objectification occurs when an individual, typically a woman, is reduced to an object for the sexual gratification of others. Some examples of sexual objectification are catcalling, rating women's appearances, ogling, sexual comments about women's bodies, and nonconsensual touch. Women frequently witness sexual objectification in their everyday lives, especially when they consume any form of media. The sexualization of women is widespread in film, magazines, television, music videos, and social media.

According to Objectification Theory by Barbara Fredrickson and Tomi-Ann Roberts, when women are exposed to sexually objectifying content or experiences, they impose an outsider's perspective onto themselves and view themselves as an object. This is known as self- objectification. Fredrickson and Roberts proposed that self-objectification could lead to depression, eating disorders, sexual dysfunction, shame, and reduced concentration in women. This research has been corroborated by numerous studies, some of which suggest that objectification leads to other detrimental effects such as increased violence against women and daily experiences of negative and self-conscious emotions. Research also discusses how the pressure for women to be impossibly thin is a driver of depression and eating disorders.

In the early 2000s, the body positivity movement gained traction on social media sites such as Tumblr and Instagram. The movement, which is a product of the fat rights movement from the 1970s, aims to spread the message that all bodies are beautiful. The movement has been beneficial for women as it helps them deconstruct their selfobjectification and provides a sense of community for them. However, the movement has been appropriated by corporations and women who meet the beauty standard. In addition, there are several different branches of body positivity now, resulting in conflict and division within the movement. Body positivity has helped minimize some of the effects of sexual objectification, but it is not the solution to the problem. The solution to the problem is teaching your students why objectification is wrong, and why they should speak out when they see it happening.

#### Lesson Plan

-

#### Title Women, not objects Subject Author Grade level Time duration Overview

| Title            | Women, not objects  |
|------------------|---|
| Subject          | Health  |
| Author           | Lauren Lloyd  |
| Grade<br>level   | 7-8th   |
| Time<br>duration | 3 class periods, each approximately 45 minutes long.  |
| Overview         | In this lesson plan, students will learn how to recognize<br>objectification, the harmful effects of objectification, and<br>how to deconstruct their objectifying attitudes. |
| Objective        | To dismantle the pervasive objectification of women in society by educating students about its detrimental impacts.   |
| Materials        | 1 handout<br>2 PowerPoint presentations (one with activity) 1 article<br>2 images<br>2 videos   |

#### **LESSON 1: What is objectification?**

Goal:

• To teach students how to recognize objectification .

Materials:

- PowerPoint
- Links to video
- "Objectification in The Media" Handout

Activity:

• True or False game

1. Have students watch What Our Kids See and ask them to share their thoughts on the video.

 "What Our Kids See" by WomenNotObjects (2:04) on YouTube. https://www.youtube.com/watch?v=yBo-G8QRpEo.

2. Show the "What is Objectification" PowerPoint presentation from beginning and ask students to discuss the question on Slide 2.

3. Play "True and False" game on Slide 3-Slide 12. Have students read the statement on the slide and decide whether the answer is true or false. Reveal the answer to the class by clicking again on the slide. If the students answered incorrectly, draw their attention to the explanation provided on the PowerPoint.

4. Have them journal about the questions on the last slide until the end of class.

5. Pass out "Objectification in the Media" assignment. Have students fill it out before the next class.

#### NAME: \_\_\_\_\_ OBJECTIFICATION IN THE MEDIA

Instructions: Pay attention to the media you consume for a day. See if you find any examples of objectification on TV, on social media, in ads, in a book, in a video game, etc.

- How many examples of objectification did you find? Why were they examples of objectification?
- 2. Where did you find these examples?

- 3. Were you surprised by the number of examples you found?
- 4. Did this activity make you realize anything about yourself/ the media/your peers/society?

#### WHAT IS OBJECTIFICATION?

"What is Objectification" PowerPoint presentation. What is Objectification.pptx

LESSON 2: Why is objectification harmful? Goal:

• To teach students about the damaging effects of objectification

Materials:

- 2 images
- 1 article
- PowerPoint presentation
- Debrief the "Objectification in the Media" assignment. Ask students to share what they found, and what they took away from the activity.
- 2. Show students the two images for this lesson.
- Have students read "How sexualization of girls creates long-term problems that harm all children" and mark the text.
  - "How sexualization of girls creates long-term problems that harm all children" by Lois M. Collins (Sept 17, 2020) from the Deseret News. https://www.deseret.com/indepth/ 2020/9/17/21432749/media-netflix-

cuties- sexualizes-girls-tv-videogames-toys-sexual-harassmentassault.

- Show students "The Harm of Objectification" PowerPoint presentation and have them take notes.
- 5. Ask students to prepare 1 discussion question and 1 potential solution to objectification for the next class.

https://www.kickstarter.com/projects/ fhsfightback/fhs-fightback-a-feministresource-kit- designed-by https://www.verywellmind.com/what-isthe-male-gaze-5118422

The Harm of Objectification

"The Harm of Objectification" PowerPoint presentation. The Harm of Objectification.pptx



https://www.kickstarter.com/projects/fhsfightback/fhs-fightback-a-feminist-resource-kitdesigned-by

LESSON 3: What can I do to stop the objectification of women? Goal:

• To encourage students to act against objectification

Materials:

- Link to Video
- Discussion questions

Activity:

Socratic Seminar

 Show students Body Positivity or Body Obsession? Learning to See More and Be More. • "Body Positivity or Body Obsession? Learning to See More and Be More" by

Lindsay Kite (16:48) from TEDxSalt Lake City. https://www.ted.com/talks/

lindsay\_kite\_body\_positivity\_or\_body\_obsession\_lear ning\_to\_see\_more\_and\_be\_more.

2. Put students in small groups for 5-10 minutes and have them discuss the solutions they came up with.

3. Hold Socratic Seminar about the discussion question and solution students brought to class.

#### Notes

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About the Author

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32. The WWII American Homefront and the **Development of** the Modern LGBT Movement in the **United States: A Case Study in Using Thematic** and Place-Based **Methodologies in** Historical

# Historical Interpretation for the National Park Service (NPS) Eliana Massey (Editor)

Faculty Mentor: Matthew Basso (History, University of Utah)

Concurrent thematic and place-based approaches improve our ability to interpret and interact with the history of the WWII home front. In conversation with NPS historians, Dr. Basso's research team developed themes that would shed new light on the World War II home front experience and thereby lead to a richer understanding of the complexities of the period. These themes include the history of sexuality, the environment, disability, and gender, as well as Native American, Filipino American, Chinese American, Mexican American, and Latine communities. By selecting these themes before conducting source searches, we were able to ensure a comprehensive and inclusive representation of relevant topics and demographics in our research. We found that a place-based approach, which was critical for our NPS goals, worked well with a thematic approach. Place-based approaches are well-received by the public. They further their understanding of the past's relevance to the present in a highly contextual setting. Our team researched the home front history of every state and territory focusing on sites in each place that revealed the new histories the NPS tasked us to illuminate. In the case of the relationship between the WWII home front and the modern LGBT (Lesbian, Gay, Bisexual and Transgender) movement in the United States, place-based history shows the importance of sites such as gay bars all over the country and how the war prompted migrations of individuals coupled with an American wartime ideology of freedom, which all too often did not apply to minority communities, shaped the practices and the geographic dispersion of the modern LGBT community.

About the Author

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# 33. Reflection on

# Undergraduate

### Research

# **Experience - Cody**

## **Howell Page**

Cody Howell Page (University of Utah)

Faculty Mentor: Dr. Kendall Gerdes (Writing and Rhetoric Studies, University of Utah)

The Undergraduate Research Opportunities Program gave me the chance to further develop my passion for writing as I focused on issues that were important to me and to the strength of my community. I have learned that one does not need to be a powerful business owner, a prestigious professor, or a politician to make a difference, rather as an undergraduate student, I can be a force for good through my writing and research. As a future law school student, I initially participated in UROP to prepare myself for the reading and writing that is so prevalent in law school, and while it has done that, my participation has changed the type of law I want to practice. As a future attorney, I want to help minority owned businesses and social entrepreneurs start companies to make a difference and solve social issues in the communities in which they live.

About the Author

Cody Page UNIVERSITY OF UTAH 34. Forgiveness: A

**New Rhetorical** 

Strategy for

**Creating an** 

# **Equitable Criminal**

### **Justice System**

Cody Howell Page (University of Utah)

Faculty Mentor: Kendall Gerdes (Writing and Rhetoric Studies, University of Utah)

With the increase in police brutality demonstrated in the killings of George Floyd, Breonna Taylor, and Tamir Rice, the criminal justice system is under the microscope of public scrutiny, and institutional change is demanded. Within the criminal justice system people of color are disproportionately affected by overly harsh administrative laws such as minimum mandatories, and racial prosecutorial rhetoric, leading to an increase in imprisonment and a decrease in equitable justice. The administrative laws responsible for the current climate within the criminal justice system were created in response to the Civil Rights movement in an effort to limit judicial discretionary power. Before the Civil Rights movement of the 1940's to the 1960's, judicial discretionary power was the means by which justice was delivered in the courts; essentially, judicial discretionary power allowed judges to use their own reasoning to determine the timeline and severity of the sentence. This method gave way to racism, as many judges used their power to favor white people while simultaneously suppressing people of color.

Post-Civil Rights movement, in an effort to rid the criminal justice system of the inequity rampant throughout, congress standardized sentencing law by instituting strict administrative laws know as minimum mandatories. With the adoption of these administrative laws, equality, at first, seemed to increase, as power was taken from judges. However, after decades of use, it is apparent that these laws may actually be more arbitrary, more violent, and more suppressive than discretionary power. Carol Steiker, Rachel Barkow, Azia Huq, and Julia Shaw, all legal scholars striving to address inequity in the criminal justice system, have advocated extensively for the abandonment of arbitrary, overly strict laws in favor of laws that force the criminal justice system to examine each case and each person individually. They claim to varying degrees that this will eliminate racial bias and increase mercy in the criminal justice system. However, if there is to be lasting and equitable change within the criminal

justice system, it requires more than changes in policy.

It is clear now, that changes in policy without a change in rhetoric failed as the state transitioned from judicial discretionary power to administrative laws - because the rhetoric driving the criminal justice system had not changed, the policies had no effect in the long term in alleviating racial biases. I use the legal research of Steiker, Barkow, Huq, and Shaw to create a new rhetorical driving force within the criminal justice system, namely the rhetorical theory of forgiveness. Building upon legal principles, the theory of forgiveness allows for an increase in discretionary power while eliminating racial bias by holding judges and prosecutors accountable for racial rhetoric and acts of inequality. Rather than basing the success of prosecutors and judges on the number of people punished and the severity of those punishments, the theory of forgiveness institutes restoration and reintegration as the new standard of success. Prosecutors and judges will now be motivated to focus on helping individuals find restoration and reintegration through individualized sentencing practices that consider the circumstances of each case. As the theory of forgiveness becomes the underlying rhetoric of the criminal justice system, actors will feel a greater sense of responsibility to individuals and their unique circumstances; in essence, each person will receive the help - not

punishment – they need to return to society. This will have long term effects in reducing the racial disparities evident within the criminal justice system.

My research advocates for the adoption of the theory of rhetoric within the criminal justice system by exploring current constitutional legal research. This research is indicative of where the criminal justice is going but fails to address more than the policy changes that must be made. However, applying rhetorical principles to the legal concepts advocated for allows one to see the criminal justice system through a new lens. I address the true issues facing the criminal justice system: the rhetoric that determines how policies are applied. With changing policies, I advocate for changing rhetoric; this cross-disciplinary research creates an environment in which true change can occur.

About the Author

Cody Page UNIVERSITY OF UTAH
**DEPARTMENT VIII** 

# **School of Medicine**

35. The Impact of

**Air Pollution** 

**Exposure on** 

**Childhood Cancer** 

**Survivors: Creating** 

and Obtaining

Feedback from

**Researchers on an** 

Informative

### Brochure

Sarah Bayardo (University of Utah); Anne Kirchhoff Pediatrics, University of Utah); Marlon Lopez (University of Utah); Karely Mann (University of Utah); and Judy Y. Ou (University of Utah)

Faculty Mentor: Anne Kirchhoff (Pediatrics, University of Utah)

#### Introduction

Air pollution is a significant public health problem that, and more than 4 in 10 Americans live in places with unhealthy levels of air pollution. Air pollution is linked to many cancer types. To inform childhood cancer survivors, their families, and caregivers about the potential negative impact of air pollution exposure, we created an informative brochure on this topic. We received feedback on our first brochure draft through an online survey sent to researchers connected to Huntsman Cancer Institute (HCI).

#### Methods

We conducted a systematic literature review on the potential risk childhood cancer survivors face when exposed to air pollution through PubMed; 22 papers were reviewed and summarized. We compiled our literature review findings into a brochure focused for parents and caregivers of young cancer survivors using concise and easy-to-understand language, and visuals appropriate to the topic. A survey was created through REDCap and sent to 37 HCI staff and researchers to get feedback on the brochure's phrasing, language, and visual appeal. The questions on our survey included demographics, familiarity with pollution science, brochure air understandability, opinions on brochure visuals, preferred

section, how ready the brochure is to be released, and feedback on how the brochure could be improved.

#### Results

A total of 17 participants completed the survey; 88.2% are females, with 70.6% having more than a 4-year college degree. We asked if the brochure was ready to be released on a scale of 1 to 10, 1 for "not ready" and 10 for "ready" the mean was 7.33. The favorite elements of the brochure were the visuals (100%) and the key messages (75%). The least favorite elements were the formatting and the colors.



88.2% learned something new from the brochure



Most (88.2%) found the brochure "not hard to understand"

#### Conclusion

Our brochure received generally positive feedback. Creating approachable ways of expressing scientific information is vital to protecting vulnerable populations such as children from air pollution. The following steps include getting feedback from parents and caregivers of survivors for future dissemination. The following steps include improving the brochure, mainly changing the color palette, title, formatting, and text length. Finally, getting feedback from parents and caregivers of survivors for future dissemination.

#### Acknowledgment

This work was supported by the Office of Undergraduate Research's Summer Program for Undergraduate Research and the National Institute of Environmental Health Sciences award R25ES031497. The content in this presentation is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. About the Authors

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Judy Ou UNIVERSITY OF UTAH 36. The Feasibility

of Using a Novel

Somatostatin

**Receptor 2** 

# **Inhibitor to Treat**

# **Type 2 Diabetic**

# Rats

Samuel Evans (University of Utah); Owen Chan (Internal Medicine, University of Utah); and Chukwuemeka Uzo (University of Utah)

Faculty Mentor: Owen Chan (Internal Medicine, University of Utah)

Loss of the ability to secrete glucagon, a hormone that is important to help raise blood glucose levels, in patients with advanced type 2 diabetes (T2D) places them at greater risk for experiencing hypoglycemia. Although the mechanism underlying this loss is currently not known, elevated somatostatin levels in poorly controlled T2D may play a role as it binds to type 2 somatostatin receptors on pancreatic alpha-cells to inhibit glucagon secretion. The current study evaluates whether a new somatostatin receptor 2 antagonist, ZT-01, can be used to help restore glucagon secretion in response to hypoglycemia in advanced T2D rats. In addition, we also examine whether ZT-01 affects metabolic parameters under hyperglycemic conditions. Sprague-Dawley rats placed on a high-fat diet and given a low-dose of streptozotocin were used to model advanced late stage T2D in humans. These animals underwent а hyperinsulinemic-hypoglycemic either or hyperglycemic clamp following the administration of ZT-01 to evaluate the effect of the drug on glucagon and insulin secretion, respectively. In response to hypoglycemia, plasma glucagon responses were almost completely absent in the T2D animals, whereas treatment with ZT-01 improved glucagon responses in the diabetic rats. Under hyperglycemic clamp conditions, we observed a significant and unexpected rise in plasma insulin levels that was accompanied by a

rise in glucagon than was observed in response to hypoglycemia. Based on the data collected, ZT-01 appears to be effective at enhancing glucagon secretion in advanced T2D rats during hypoglycemia but surprisingly, it also stimulated insulin secretion under hyperglycemic conditions, which may prove to be an advantageous therapy in maintaining metabolic control in T2D.

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# 37. Acculturation,

# Acculturative

# Stress, and Suicidal

### **Behavior Among**

# **Hispanics in the US**

Naomi Guerrero Reyes (Westminster College) and Erin McGlade (Psychiatry, University of Utah)

Faculty Mentor: Erin McGlade (Psychiatry, University of Utah)

Suicidal behaviors (e.g., suicidal ideation and attempts) among Hispanics in the US have been increasing since the 2000s (Silva & Van Orden, 2017). Two factors that are causing an increase in suicidal behaviors are acculturation and acculturative stress. Acculturation is defined as the process of adapting to a new culture (Mody, 2007) while the term

acculturative stress has been defined as the psychological impact of adaptation to a new culture (Smart & Smart, 1995). language comprehension, Discrimination. poor low socioeconomic status, family conflict, and low levels of support are some of the acculturative stressors that contribute to suicidal behaviors (Haboush-Deloye et al., 2015). The purpose of this review is to summarize the studies examining suicidal behaviors associated with acculturation and acculturative stress among Hispanics. Many of the studies in this review have found significant relations between suicidal behavior, acculturation, and acculturative stress. However, additional research and public health interventions are needed to better understand and address why acculturation and acculturative stress are affecting the mental health of the Hispanic community in the US. One major study that can provide insight to these associations is the nationwide Adolescent Brain Cognitive Development (ABCD) study (including the Diagnostic Neuroimaging, University of Utah site), which is a 10-year longitudinal study that includes variables on suicide behavior and acculturation in youth. Future directions for research and interventions on acculturation, acculturative stress and suicide behaviors also will be explored.

**Keywords**: acculturation, acculturative stress, suicide, suicide ideation, suicidality, suicide behavior, suicide attempt, Hispanic, Latino, Latina, Mexican, Central American, South American.

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Range 275

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**Do You Feel?** 

Emotion

# **Regulation in**

# AMCI and the

# **Aging Brain**

Ellie Han (Vanderbilt University); Joseph Kim (Psychiatry, University of Utah); Vincent Koppelmans (Psychiatry, University of Utah); Scott Langenecker (Psychiatry, University of Utah); and Sara Weisenbach

Mentor: Joseph Kim (Psychiatry, University of Utah)

#### Abstract

Reappraisal-based emotion regulation refers to a cognitive process used to willfully control the intensity of emotions we experience. Previous literature suggests that cognitive decline may reduce the proportion of successful reappraisers in MCI patients. This study aims to compare emotion regulation in healthy, neurologically normal older adults and those with memory impairments (amnestic Mild Cognitive Impairment or aMCI). We hypothesized that the aMCI group will show weaker emotion regulation effectiveness as well as worse memory and thinking skills. Older adults, aged 55-79, received a neuropsychological assessment of multiple cognitive domains including memory, and executive functioning. All participants also completed an Emotion Regulation Task (ERT) while undergoing a brain scan (functional Magnetic Resonance Imaging or fMRI). The ERT required participants to maintain, or reappraise their negative emotions while viewing negative (e.g., gore, violence, or contamination), and images subsequently rate how negative they were feeling. A linear mixed effects model was conducted to examine the relationship between age, group and instruction with ERT negativity ratings. As expected, the aMCI group performed significantly worse in the neuropsychological assessment. A Linear Regression showed that there is a significant relationship between negativity rating following "reappraise" and performance on Trail Making Test B. The linear mixed effects model showed that age, group, and instruction all significantly predicted negativity ratings. Furthermore, a paired sample ttest within each group showed that there was a significant difference in the response values following the instructions "maintain" vs "reappraise" in the healthy control group (t=-2.25, df=14, p=0.041) but not the aMCI group. Despite the

small sample size, this study demonstrates the need for subsequent studies on the relationship between cognitive decline in aMCI and emotion regulation.

### Introduction

### **Emotion Regulation**

Gross defines emotion regulation as "the process by which individuals influence which emotions they have, when they have them, and how they experience and express these emotions" [1]. Understanding emotion regulation is important because it serves as a fundamental framework necessary to improve or suppress simultaneously or subsequently performed tasks [2]. For example, previous research has demonstrated that implementing successful emotion regulation strategies in life was closely linked to overall well-being and even financial success [3]. Especially, the cognitive reappraisal emotion regulation technique, or "construing an emotioneliciting situation in different terms in order to alter its emotional impact" [4] has received significant attention from scholars.

### aMCI

With dementia being recognized as a serious public health concern [5], it is important to examine patients in prodromal stages of dementia, such as "Mild Cognitive Impairment" or MCI. MCI is diagnosed in individuals with "self- or informantreported cognitive complaint(s), objective cognitive impairment on formal examination and relatively intact activities of daily living" [6]. Amnestic Mild Cognitive Impairment (aMCI) is a dominant subtype of MCI in which memory loss is the predominant symptom [7]. The memory impairment in aMCI is caused by the atrophy of the hippocampus, amygdala, posterior cingulate, and superior parietal lobule observed in patients with aMCI [8]. In addition to memory loss, according to the Mayo Clinic Study of Aging (MCSA), affective and emotional dysregulation symptoms such as anxiety and depression are common in people with MCI, with apathy, agitation, and irritability especially higher in people with aMCI [9].

### aMCI and the Aging Brain + Emotion Regulation

To better understand the relationship between cognitive impairment and emotion regulation, Xiao et al. compared MCI and Healthy adults' (age 55-86) abilities to "reappraise" negative images [10]. The results from this study reflected that cognitive decline reduced the proportion of successful reappraisers in the MCI group [10]. These results suggest that MCI patients might have difficulty reinterpreting or detaching/ distancing oneself from a stimulus [4] due to cognitive decline observed in aMCI.

Cognitive decline observed in aMCI is associated with prominent deficits in learning and memory that is above and beyond what is expected in normal aging. Some degree of cognitive decline is normal in aging. For example, fluid intelligence – the ability to reason and problem-solve in novel situations have been known to decline with older age. Similarly, previous studies show processing speed, executive functioning, aspects of memory and psychomotor abilities [11] decline in older adults. However, with cognitive decline that follows aMCI, there is a notable decline in learning and memory characterized by weak learning curve, rapid forgetting, poor recognition indicative of poor retention of information, and increased susceptibility to interference effects on episodic memory tasks [12].

### **Our Study**

Previous work has shown that cognitive decline in aMCI patients limit their cognitive reappraisal emotion regulation.

However, the connection between aMCI patients' neuropsychological cognitive abilities, specifically learning and memory, and emotion regulation needs more research. Therefore, the purpose of this study is to compare emotion regulation in healthy, neurologically normal older adults and those with memory impairments (amnestic Mild Cognitive Impairment or aMCI).

### **Materials and Methods Participants**

Potential participants were provided a description of the study and were administered a standard telephone screen that included questions about demographics, medications, medical problems, presence of metal in the body and a screener for depressive symptoms and dementia. Eligible participants' informed consent was obtained through the University of Utah's Huntsman Mental Health Institute through video conferencing. After consent, a semi-structured psychodiagnostic interview (Structured Clinical Interview for DSM-5, or SCID-5) and a set of cognitive screening measures were administered to determine study eligibility, psychiatric history and medical history. A total of 19 participants (mean age, 69±6.2) completed all parts of the study, including 15 Healthy Control (HC) and 4 aMCI. Participants meeting the following criteria were included: (1) sufficient visual and hearing capacity to follow instructions and engage in cognitive (2) ability to abstain from assessment; benzodiazepines and opiates at least 24 hours prior to study visits; (3) no history of recent severe medical illness, brain/neurological disorders; and (4) no physical problems that are inappropriate for fMRI scan. In addition to above criteria, the aMCI

patients also had to meet criteria for amnestic MCI based upon the "Jak and Bondi" criteria which included two or more of the delayed recall measures from the three memory tests falling below the 1 standard deviation from the mean [13].

### Neuropsychological Assessment

eligible Fully participants completed а virtual neuropsychological assessment to assess the cognitive functions of all participants. The Animal Fluency Test measured language and executive functions, and part B of the Trail Making Test (TMT-B) measured set-shifting as part of executive functioning. The Hopkins Verbal Learning Test-Revised (HVLT-R) measured verbal learning and memory, and the Brief Visuospatial Memory Test-Revised (BVMT-R) measured visuospatial learning and memory. After the neuropsychological assessments, self- report questionnaires were completed by participants.

### **Functional Magnetic Resonance Imaging**

During the second study visit, participants completed a functional Magnetic Resonance Imaging (fMRI) brain scan at the Utah Center for Advanced Imaging Research at The University of Utah. In the scanner, under the Emotion Regulation Task (ERT), participants were asked to follow one of three different instructions, Look, Maintain or Reappraise. The Look

instruction required participants to view a neutral picture and respond whereas the Maintain instruction required participants to be aware of what they naturally feel when looking at a negative (e.g. gore, disgusting) image. The Reappraise instruction required participants to use a thoughtful strategy to reduce negative feelings brought on by looking at an unpleasant picture. Following the instructions, participants then rated how "negative" they were feeling.

### Analysis

One Sample T-tests were conducted to examine the relationship between two groups' neuropsychological assessment performances measured in scaled t-scores. A Linear Mixed Effects Model was conducted to examine the relationship between age, group, ERT negativity rating response value, and group's interaction with ERT negativity rating response value. In this analysis, negativity rating following "maintain" was set as reference and negativity rating following "look" and "reappraise" were each compared to the reference. Further, a Paired Sample T-test within each group's ERT negativity rating response value was conducted to show significant difference between ERT negativity rating response value following two different instructions. Later, a Linear Regression Model was conducted to compare TMT-B performance and ERT negativity rating response value following "reappraise."

Results

### Neuropsychological Assessment

Table 1. Neuropsychological Assessment Results by Test and Group

|                        | aMCI Mean   | HC Mean     | two-rample-r | df. | p-value |
|------------------------|-------------|-------------|--------------|-----|---------|
| Animal<br>Fluency Test | 43.6 (14.6) | 59.2 (10.6) | 2.54         | 20  | 0.031*  |
| TMT-B                  | 48.3 (6.92) | 54.4 (10.9) | 1.56         | 20  | 0.070   |
| HVLT-R                 | 33.9 (13)   | 55.4 (5.18) | 4.24         | 20  | 0.0040* |
| BVMT-R                 | 41.9 (9.34) | 56.7 (7.45) | 3.70         | 20  | 0.0043* |

\* The mean difference is significant at the 0.05 level.

The aMCI group performed significantly worse than the

HC Test, HVLT-R and BVMT-R. Moreover, there was a trend toward between-group difference in TMT-B performance.

fMRI Emotion Regulation Task (ERT)

Table 2. Emotion Regulation Task Linear Mixed Effects Model

|      | Maintain<br>>Look |    | 1       | Reappraise ><br>Look | 1  |         | Reappraise ><br>Maintain |    |         |
|------|-------------------|----|---------|----------------------|----|---------|--------------------------|----|---------|
|      | 2                 | df | p-value | 1                    | ď  | p-value | 2                        | df | p-value |
| aMCI | 12.07             | 3  | 0.0012* | 14.03                | 3  | 0.0008* | -1.23                    | 3  | 0.31    |
| HC   | 9.16              | 14 | <.0001* | 9.12                 | 14 | <.0001* | -2.25                    | 14 | 0.041*  |

\* The mean difference is significant at the 0.05 level.

The Linear Mixed Effects Model showed a significant effect of age, group, and instruction on ERT negativity rating response value. Older age was associated with generally higher negativity rating and HC group was associated with generally lower negativity rating. When compared to ERT negativity rating response value following instructions to "maintain," ERT negativity rating response value following instructions to "look" and "reappraise" were generally lower.

Table 3. Emotion Regulation Task Matched Pairs: Instructionsand Response

|                    | Sum of<br>Squares | Mean of<br>Squares | NumDF | DenDF | Fvalue | Pr(>F)    |
|--------------------|-------------------|--------------------|-------|-------|--------|-----------|
| Age                | 1.15              | 1.15               | 1     | 16    | 5.11   | 0.038*    |
| Group              | 1.01              | 1.01               | 1     | 16    | 4.50   | 0.050*    |
| Instruction        | 26.12             | 13.06              | 2     | 34    | 58.21  | 1.05e-11* |
| Group: Instruction | 0.21              | 0.11               | 2     | 34    | 0.47   | 0.63      |

\* The mean difference is significant at the 0.05 level.

The Paired T-Test showed a significant difference between ERT negativity rating response value following any two different instructions in the HC group. For example, in the HC group, ERT negativity rating response values are highest following "maintain" and then "reappraise" and "look" respectfully. However, there is no significant difference between ERT negativity rating response value following "reappraise" and "maintain" in the aMCI group.



Fig. 1 Reappraise ERT Response vs. TMT-B

There is a significant correlation between ERT negativity rating response value following "reappraise" and TMT-B performance for the entire sample (slope = 5.002, N = 18, P = 0.0016,  $\bigotimes^2 = 0.452$ ).

#### Discussion

Reappraisal based emotion regulation can allow someone to willfully control the intensity of emotions they experience. With previous literature stating emotion dysregulation as a symptom of aMCI, our study is collecting additional data to further understand the relationship between cognitive impairment and emotion dysregulation in aMCI. The neuropsychological assessment revealed aMCI's significantly worse performance in executive functioning, and verbal, and visuospatial learning and memory relative to HCs. aMCI's worse performance in HVLT delayed recall and BVMT delayed recall is consistent with previous findings of delayed recall related memory impairments as aMCI's dominant symptom.

The lack of significant difference in aMCI's ERT negativity rating response between the Maintain and Reappraise instructions may be suggestive of reappraisal-based emotion regulation difficulties as a symptom of aMCI. However, the significant difference in aMCI's ERT negativity rating response value following instructions to "maintain" or "look" shows that aMCI patients are emotionally reactive to negative stimuli. The significant relationship between ERT negativity rating response value following "reappraise," and performance on further analysis. TMT-B requires The brain-behavior relationship between "reappraise" and executive functioning can be explored in relation to the functioning of the ventrolateral prefrontal cortex or the inferior frontal gyrus (IFG).

### Conclusion

Though the results from this study are still preliminary due to ongoing data collection efforts, these findings are aligned with prior studies suggestive of convergence between cognitive and affective control challenges experienced by individual who suffer from memory impairments associated an aMCI diagnosis. Further analysis with increased sample size will be necessary to better characterize the relationship between cognitive decline and emotion regulation in aMCI. A solid understanding of atrophy in aMCI through detailed analysis of the neuropsychological assessments and the neuroimaging data will also inform the mechanism that underlie the putative relationship between cognition and emotion in aMCI. Further data collection and analyses resulting from this study will help pave the way for identifying novel treatment for emotion dysregulation in aMCI. Future work should include further investigation into the brain-behavior relationship in aMCI, especially in the ventrolateral prefrontal cortex and its relation to emotion regulation.

#### Acknowledgement

I would like to express my sincere gratitude to my supervisory team, Joseph Kim, Ph.D. and Vincent Koppelmans, Ph.D. This work was supported by funding from the Office of Undergraduate Research's Summer Program for Undergraduate Research at the University of Utah awarded to Ellie Han. This work was also supported by the National Institute of Mental Health R01 awarded to Sara Weisenbach.

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39. A Pilot Study

**Replicating Foot** 

and Ankle

**Kinematics and** 

**Kinetics Using a** 

# **Custom Robotic**

# Simulator

Jordy A. Larrea Rodriguez (University of Utah); Amy Lenz (Orthopaedics,University of utah); and Anthony H. Le (University of Utah)

Mentor: Amy Lenz (Orthopaedics, University of Utah)

Conventional in vivo foot and ankle models with skin-

mounted retroreflective markers are encumbered by inaccuracies related to skin-motion artifact and poor rigid body assumptions, where kinematic contributions of individual bones are lost [1, 2]. These assumptions lead to inaccurate descriptions of specific joint motions observable in lower extremity prosthetic designs and foot and ankle surgery. biplane fluoroscopy studies Although, *in vivo* have demonstrated more accurate individual bone motion tracking, these studies are limited by small sample sizes, the heterogeneity of the typical human population, and expensive equipment. Robotic simulators allow for direct kinetic and kinematic measurements in vitro while eliminating the heterogeneity of in vivo studies, where physiology and morphology vary [1]. The purpose of this pilot study is to develop a simulator for collecting in vitro kinetic and kinematic data during plantar-/dorsiflexion, inversion/eversion, and internal/external rotation. Three cadaveric male right tibial plateau-to-toe tip specimens will be procured. Infrared motion tracking markers will be mounted directly to the tibia, fibula, talus, calcaneus, cuboid, and navicular bones. A six-axis robotic manipulator will position the specimen over three load cells to measure forces and moments under the first and fifth rays and heel while manipulating the foot and ankle through the prescribed motions. Kinetic and kinematic data will be collected in sync using a custom LabVIEW program. Findings from this study will provide a better understanding of the tibiotalar. subtalar, calcaneocuboid, talocrural, and talonavicular joint motions; thus, informing future implant development and surgical treatment for the foot and ankle.

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40. Creating and

Obtaining

Feedback on a

**Childhood Cancer** 

Environmental

**Health Brochure:** 

**Results from** 

### **Researcher Survey**

Marlon Lopez (University of Utah); Anne Kirchhoff Pediatrics, University of Utah); Sarah Bayardo (University of Utah); Karely Mann (University of Utah); and Judy Y. Ou (University of Utah) Faculty Mentor: Anne Kirchhoff (Pediatrics, University of Utah)

#### Introduction

Approximately 1 in 285 children in the U.S. will be diagnosed with cancer before their 20th birthday<sup>1</sup>. Many parents and caregivers of children with cancer have little to no knowledge of how the environment could be an attributing factor on the development of cancer<sup>2</sup>. Our goal is to inform the public about how environmental carcinogens may affect the risk of cancer by creating a brochure on this topic, mentioning carcinogens found in daily life as well as prevention methods for parents and caregivers of childhood cancer survivors. We received feedback on the brochure through an online survey sent to researchers connected to Huntsman Cancer Institute (HCI) with questions that would provide us with quantitative as well as qualitative data.

#### Methods

We conducted systematic literature а review on environmental carcinogens in research publications such as PubMed and Science direct; in total, 11 papers, relating to environmental carcinogens, were reviewed, and summarized. We then compiled a brochure for caregivers of young cancer survivors that would use concise and lay language. We created and sent out a survey through REDCap to HCI researchers. The survey questions included demographics, comprehensibility, if the information was educational, their preferred section, and how ready the brochure is to be released.

Results: The survey was sent to 26 researchers; 19 had usable survey information. Of these, 76.5% of had more than a 4-year college degree; 73.7% were somewhat to very familiar with the field of environmental health. Around 21.1% said they knew a lot about environmental health. The majority (89.5%) found the language of the brochure "not hard to understand" and 78.9% of participants reported learning something new. Most participants indicated the content on how to reduce exposure as their favorite (64.7%). On a scale from 1-10 for whether the brochure was ready for dissemination (1=not ready; 10=completely ready), the mean was 6.29.







#### Conclusion

The brochure received positive feedback from participants. Participants expressed mostly favorable opinions on the brochure's contents. The participants mostly agreed that it does not include difficult language, and that they absorbed new information, exhibiting positive as well as constructive opinions on the various section of the brochure. Limitations in this research included not having the sufficient time to send the survey out to more participants with a more diverse background, in education and knowledge. Receiving feedback from our target audience would be most valuable since it was meant for them. Next steps include getting feedback from parents and caregivers of survivors as well as further peer revisions and edits for future dissemination based on the feedback that we received. Creating approachable ways of communicating scientific information is vital to protecting vulnerable children from environmental carcinogens.

#### Acknowledgment

This work was supported by the Office of Undergraduate Research's Summer Program for Undergraduate Research and the National Institute of Environmental Health Sciences award R25ES031497. The content in this presentation is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Range 297

Karely Mann UNIVERSITY OF UTAH

Judy Ou UNIVERSITY OF UTAH 41. Examining the

Temporal

Relationship

**Between Air** 

**Quality Trends and** 

Glycemic

**Outcomes Among** 

Patients with Type

# 2 Diabetes Mellitus

Catherine Petersen (University of Utah); Ramkiran Gouripeddi (Biomedical Informatics, University of Utah); Sejal Mistry (University of Utah); and Julio C. Facelli
#### (Biomedical Informatics, University of Utah)

Faculty Mentor: Ramkiran Gouripeddi (Biomedical Informatics, University of Utah)

#### Abstract

Type 2 diabetes mellitus (T2DM) is a chronic condition caused by insulin resistance and metabolic dysfunction. Approximately 37 million individuals in the United States have T2DM.<sup>6</sup> Long term exposure to air pollution is thought to increase insulin resistance and impair glucose metabolism due to oxidative stress and inflammation.<sup>4</sup> Particulate matter with an aerodynamic diameter  $\leq 2.5 \ \mu m$  (PM<sub>2.5</sub>), more specifically its ultrafine component, can cross the pulmonary alveolar membrane and direct inflammatory effects on target organs.<sup>4</sup> While several studies have identified a relationship between  $PM_{2.5}$  concentrations and onset of  $T2DM^{1,2,4}$ , few studies have examined the role of air pollution on glycemic outcomes after T2DM diagnosis. There is a possibility of air pollution worsening glycemic control and metabolic dysfunction and contribute to poor glycemic outcomes. Therefore, the objective of this research was to evaluate the relationship between temporal trends in PM2.5 concentrations and glycemic outcomes among patients with T2DM in Davis, Utah, and Salt Lake Counties in Utah. Electronic medical record data from EPIC for 143,434 patients with an eligible ICD-10 diagnosis code for T2DM from 2010-2022 were selected for analysis. PM<sub>2.5</sub> concentrations were extracted from the EPA's Air Quality System in Davis, Utah, and Salt Lake counties in Utah.

The date of a patient's initial diagnosis was found and visually assessed with one-year trends in  $PM_{2.5}$  concentrations. Analysis is ongoing and it is hopeful that the results of this study will elucidate the role of  $PM_{2.5}$  concentrations on glycemic outcomes in patients with T2DM and may inform public health interventions to minimize air pollution and encourage better outcomes for individuals with T2DM.

#### Introduction

Type 2 diabetes mellitus (T2DM) is a chronic condition caused by insulin resistance and metabolic dysfunction<sup>4</sup>. Diabetes affects roughly 37 million people, with T2DM accounting for roughly 90-95% of diabetes prevelence<sup>6.</sup> T2DM is associated with many diabetes-related complications, including heart disease, vision loss, and kidney disease.<sup>7</sup>

The potential risk factor of interest for this study is exposure to air pollution. As illustrated in Figure 1, the ultrafine component of particulate matter with an aerodynamic diameter  $\leq 2.5 \ \mu m (PM_{2.5})$ , can cross the pulmonary alveolar membrane and is thought to increase insulin resistance and impair glucose metabolism due to oxidative stress and inflammation on target organs.3 Although several past studies have identified a relationship between PM<sub>2.5</sub> concentrations and onset of T2DM<sup>1,2,4</sup>, few studies have examined the role of air pollution on glycemic outcomes after T2DM diagnosis. Therefore, the objective of this research was to evaluate the relationship between temporal trends in PM<sub>2.5</sub> concentrations and glycemic outcomes among patients with T2DM in Davis, Utah, and Salt Lake Counties in Utah.



Figure 1: Schematic representation of process starting with air pollution exposure leading to development of T2DM

Figure 1: Schematic representation of process starting with air pollution exposure leading to development of T2DM

#### Methods

In this study, electronic medical records from EPIC at the University of Utah from 2000-2021 were obtained and processed for 143,434 patients with an ICD-9 or ICD-10 diagnosis code for T2DM. Patients with an address in the state of Utah and with a zip code in Salt Lake, Davis, or Utah counties were included. PM2.5 concentrations were obtained from monitors in the EPA Air Quality Systems. The daily average concentration of PM2.5 were obtained under local conditions from 2000-2021 to use as an approximation of air quality levels in Salt Lake, Davis, and Utah counties. The Haversine distance from each included patient to each monitor of interest was calculated using the patient and monitor latitude and longitude. Next, the three nearest monitors for each patient were identified. Patients with an address more than 45 km away from the nearest EPA monitor and patients lacking an eligible ICD-9 or ICD-10 code signifying a T2DM diagnosis were excluded. The date of first diagnosis with an eligible ICD-9 or ICD-10 code for type 2 diabetes was extracted from EPIC electronic medical records and the date one year after diagnosis was obtained. All available PM2.5 average daily concentrations were found at the nearest monitor to a patient for 365 days after they were diagnosed with T2DM. Patients with fewer than 100 PM<sub>2.5</sub> measurements in the year following diagnosis were excluded. Missing PM<sub>2.5</sub> concentrations were imputed using linear interpolation and PM<sub>2.5</sub> concentrations were scaled using a mean variance scalar. After cleaning the subject data to meet determined inclusion/exclusion criteria, as depicted in Figure 2, a total of 33,968 patients were included in the study. From these patients, we extracted the mean age, gender ratio, number with pre-HbA1c and post-HbA1c information available, how many had increased HbA1c after 1 year, how many had decreased HbA1c after 1 year, and how many had no change in HbA1c after 1 year. Along with this, we found how many were prescribed with an antidiabetic medication after diagnosis, and which type of medication they were prescribed to.



Figure 2: Inclusion and exclusion criteria for subjects included in study

Figure 2: Inclusion and exclusion criteria for subjects included in study

Exposure profiles of daily PM2.5 concentrations 1-year after T2DM diagnosis were identified using shape-based K-means time-series clustering<sup>5</sup>. K-means clustering is a type of unsupervised machine learning method that identifies subgroups of data. Shape-based time-series clustering methods use cross-correlation to identify clusters in time-series data<sup>3</sup>.

A K-means shape-based time-series clustering algorithm was trained to identify temporal  $PM_{2.5}$  exposure profiles using a maximum iteration of 10, initialization of 1, and clusters of 2-20. Clustering performance was evaluated by calculating the inertia for each cluster and the elbow method was used to identify the optimal cluster number. This work was done using Python 3.9.12. Packages used were pandas 1.4.2, numpy 1.22.3, geopy 2.2.0, and seaborn 0.11.2.

#### Results

| Variable    | Units   | Value          |
|-------------|---|----------------|
| Ago         | mican age + s.d.  | 63.8 ± 16      |
| Gender      | % fernale (n)   | 48.8% (16,909) |
|             | % male (s)  | 50.2% (17,057) |
| HhAle       | % patients with pre-HbAlc & post-HbAlc data available (n)                       | 18.1%-(6,143)  |
|             | % patients with decreased EBA3e after 1-year (e)                                | 67.6% (4,153)  |
|             | % patients with increased HbA.lc after 1-year (n)                               | 28.15(1,727)   |
|             | % patients with no change in HbA1z after 1-year (n)                             | 4.3% (263)     |
| Medications | % patients with anti-diabetic medication prescription at diabetes diagnosis (n) | 68.5% (22,236) |
|             | % patients with Bigsanides prescription (n)                                     | 49.7% (16,888) |
|             | % patients with Insulin prescription (n)  | 21.1%(7.162)   |
|             |   |                |

Table 1: Population breakdown of subjects included in analysis

Table 1: Population breakdown of subjects included in analysis

As seen in Table 1, of the 33,968 subjects included in analysis, the mean age was 63.8 years old, with a standard deviation of 16 years. 49.8% (16,909 subjects) of the subjects analyzed were female and 50.2% (17,057 subjects) were male. Of all the subjects included, 18.1% (6,143 subjects) had pre-HbA1c data available for six months before or three months after T2DM diagnosis and post-HbA1c data available for six months after a year post diagnosis. Of the patients with available HbA1c data, 67.6% (4,153 subjects) had a decrease and 28.1% (1,727 subjects) had an increase in HbA1c after one year, while 4.3% (263 subjects) showed no change. Of all subjects included, 65.5% (22,236 subjects) were prescribed with an anti-diabetic medication at the time of diagnosis. Of all subjects included, 49.7% (16,888 subjects) were prescribed with Biguanides and 21.1% (7,162 subjects) were prescribed with Insulin.



Figure 3: Elbow method of inertia scores identifies 12 optimal clusters

Figure 3: Elbow method of inertia scores identifies 12 optimal clusters

To determine the optimal number of clusters, the inertia score was calculated for each cluster in our model. The elbow method was then used to determine the optimal number of clusters for analysis. To do this, the inertia score was plotted against number of clusters where it was determined that rate of decline was roughly exponential until K = 12, where it became more linear. Using this information, we determined that 12 clusters would be used for analysis.



Figare 4: Personalized air pollution exposure profile for patients diagnosed with T2DM in Salt Lake, Davis, and Utah counties in the year following diagnosis.

Figure 4: Personalized air pollution exposure profile for patients diagnosed with T2DM in Salt Lake, Davis, and Utah counties in the year following diagnosis.

Shape time-series clustering with 12 clusters were plotted (Figure 4). Cluster size ranged from n = 626 to n = 6402. Groups were clustered together based on similar features shared. For each cluster, the scaled PM<sub>2.5</sub> concentration in  $\mu$ g/m3 was plotted against 365 days after the date of T2DM diagnosis. This created a visual representation of air quality trends for one year for each group of patients. We identified variability in air pollution types for each cluster of patients. For some patients, PM<sub>2.5</sub> concentration is at a relatively low level for

most of the year with dramatic spikes signifying suddenly bad air quality (note which clusters this fits with). For others, there were multiple peaks, while others fluctuated dramatically throughout the duration of the year (note which cluster this describes). Overall, the highest scaled  $PM_{2.5}$ concentration observed was just above 8 µg/m3 and the lowest scaled  $PM_{2.5}$  concentration observed was close to -2 µg/m3.

#### Discussion

The purpose of this study was to evaluate the relationship between temporal trends in  $PM_{2.5}$  concentrations and glycemic outcomes among patients with T2DM in Davis, Utah, and Salt Lake Counties in Utah. This process allowed is to identify personal profiles of daily  $PM_{2.5}$  exposure for included patients in the year after they were diagnosed with type 2 diabetes.

This study was unique when compared to other research has done because it is the first study to characterize temporal trends in  $PM_{2.5}$  concentration after a type 2 diabetes diagnosis using data-driven methods. This is important because it allows us to develop a deeper understanding of how air pollutant exposure can potentially be a risk factor for uncontrolled or worsening glycemic outcomes.

Some limitations include a lack of access to data on indoor air quality. This is an issue because most people spend a significant amount of time indoors, where exposure to pollutants can be quite different than it is outside. For the purposes of this study, it was assumed that available EPA data could be used to approximate the general air pollution that a patient was exposed to in the year following diagnosis. The study was also limited by the inability to generate a personal air pollution profile for patients lacking an address. This could have skewed the results to favor information about a potentially more affluent subset of the population of the counties of interest, leaving out information about particularly vulnerable individuals. Due to the nature of the demographic information available, for patients with addresses, only one address was available to be used when generating exposure profiles. This means that there is a possibility that many of the patients moved or resided at a different address than was listed in the year after they received their T2DM diagnosis. Due to this, there is a potential that the PM<sub>2.5</sub> concentrations that a patient was actually exposed to were different to those estimated for this study. In the study, PM<sub>2.5</sub> concentrations in the year preceding the onset of diabetes were not found due to time constraints, which affects the ability to correlate exposure to pollutants with glycemic outcomes.

Future steps to be taken for this study include an analysis of HbA1c measurements in the time period in question. This will allow us to observe how blood sugar levels fluctuate over time and to identify any potential pattern in relation to exposure to pollutants. To further expand on this study, it would also be beneficial to expand the time frame to include both time prior to diagnosis and more than one year after diagnosis. This will allow us to create more comprehensive individual exposure profiles and develop a deeper understanding of the role of PM<sub>2.5</sub> exposure in predicting glycemic outcomes. Further analysis is also needed to determine the role of type of air pollution exposure throughout the year. For example, for some groups, air quality was relatively consistent for most of the year following diagnosis, with several dramatic spikes, while for other groups, it fluctuated dramatically. Further analysis could help determine the effects of different exposure types throughout the year. Another direction to take this work in could be to incorporate information on

various covariates known to influence diabetes onset and outcomes into the study. These covariates include demographic information such as age, race, gender, and BMI or medications being taken. Incorporating these covariates would allow us to develop a more nuanced understanding of the interplay of more of the factors that affect glycemic outcomes among patients with T2DM.

#### Acknowledgements

This work was supported by funding from the Office of Undergraduate Research's Summer Program for Undergraduate Research at the University of Utah awarded to Catherine Petersen. The study was approved by University of Utah Institutional Review Board: IRB 00134728. This research was supported by the National Library of Medicine (T15LM007124), the National Institute of Diabetes and Digestive and Kidney Diseases (F30DK134113), and the National Center for Advancing Translational Sciences (UL1TR002538). All analysis was done in Protected Environment of the Center for High Performance Computing, partially funded by the NIH Shared Instrumentation Grant 1S10OD021644-01A1.

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### 42. Reflection on

### Undergraduate

### Research

### **Experience** -

### **Monica-Rea Owens**

Monica-Rea Owens (University of Utah)

Faculty Mentor: Scott Collingwood (Pediatrics, University of Utah)

My SPUR [Summer Program for Undergraduate Research] experience went above and beyond my expectations. Not only did I gain valuable research experience, but I was able to get connected to more research opportunities and get introduced to people that I can lean on for guidance and mentorship in the future.

About the Author

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### 43. Interventional

### Assessment of Air

### **Quality on**

### Low-Income

### **Homes in Salt Lake**

### County

Monica-Rea Owens (University of Utah); Camden Alexander (University of Utah); Scott Collingwood (Pediatrics, University of Utah); and Marco Lorenzo Allain (University of Utah)

Faculty Mentor: Scott Collingwood (Pediatrics, University of Utah)

### Abstract

Salt Lake Valley is notorious<sup>1</sup> for its air pollution, which occurs when harmful matter enters the atmosphere. Our air pollution consists of particulate matter<sup>2</sup> (PM<sub>2.5</sub>), ozone<sup>3</sup>, and arsenic<sup>4</sup>, which come from vehicles, homes, and industrial emissions. Poor air quality has been linked to adverse health outcomes<sup>5</sup>, such as higher rates of cancer, heart disease, stroke, and respiratory diseases. The study's objective is to assess whether in-home interventions reduce air pollution and improve participant health. Methods include collecting air quality data within participant homes and implementing home improvements and renovations, including but not limited to installing HVAC systems, replacing swamp coolers with central AC, installing HEPA filters, and reinforcing shelter features. The study is in its elementary stages. Therefore, results have not been finalized.

Utahns have consistently cited air quality as a top concern<sup>6</sup> and have reason to be considering Utah's historic<sup>7</sup> air quality problems. Throughout the year, Utah's consistent pollutants come from vehicle exhaust, industrial processes, and home and business emissions, although the seasons offer different pollutants. In summer, wildfire smoke<sup>8</sup> and ozone levels are pollutants that lower Utah's air quality to some of the worst<sup>9</sup> in the world. And in winter,  $PM_{2.5}$  levels and the inversion<sup>10</sup> from the mountains adds to the poor air quality. This study hopes to reconcile Utah's poor air quality with implementable solutions tailored to Utah citizens, specifically Salt Lake County occupants. The Green and Healthy Homes Initiative<sup>11</sup> is a national organization aimed at providing home improvements and housing strategies to create safe, healthy, and energy-efficient housing for lowincome families. The Salt Lake County study serves as a pilot interventional research project with the home acting as the research entity. Salt Lake County funds<sup>12</sup> the initiative, providing the resources and compensation needed to carry out necessary home interventions. The study intends to increase awareness of methods to reduce air pollution and improve public health, which could serve as advocation for policy change at the state level.

#### Methods

Ten low-income homes were recruited through the Green and Healthy Homes Initiative and enrolled in the Salt Lake County research study. After recruitment, calibrated air quality sensors are deployed into participants' homes, and a pre-intervention assessment of air quality is conducted to serve as a baseline reading. Air quality sensors will then provide a summary of exposures to the participants that can be used to determine areas for home improvements and/or interventions. Possible interventions include installing proper drainage, detectors, and energy-efficient pollutant appliances, reducing clutter, improving ventilation systems, eliminating pests and pest openings, repairing and cleaning homes, and reducing the usage of dangerous cleaning products. Salt Lake

County will provide the resources and compensation needed to complete the home improvements and interventions. Upon completion of the study, a post-intervention assessment will be conducted, and the air quality sensor data will be analyzed to determine if interventions were successful in improving air quality and environmental health outcomes.

#### AIR SENSOR CALIBRATION

air quality sensors that The are installed in participants' homes are low-cost sensors calibrated to a high-quality DustTrak monitor. A mixture of ammonium nitrate and deionized water is aerosolized by an atomizer and dried in a chamber of silica beads. Once the ammonium nitrate is airborne, it simulates the fine particulate matter mass such as  $PM_{2.5}$ . The particulate matter is then pulled through a spiral chamber via the gas flow allowing the particulate matter to hit the lowcost sensors stationed at the bottom of the chamber. The low-cost sensors measure PM<sub>2.5</sub> at varying concentrations that the DustTrak monitor has set. Once the calibration session is concluded, the values that are recorded by the DustTrak monitor and the low-cost sensors are linearly modeled, and a best-fit line is applied to represent the calibration curve. Calibrating the low-cost sensors is an affirmative step that allows for precise and accurate measurements of air quality in participants' homes.

#### Limitations

The Salt Lake Valley experiences adverse air pollution with inversion most prominent<sup>10</sup> in winter, contributing to high PM<sub>2.5</sub> levels. Limitations of this study are the possibility of pre-intervention assessments conducted during winter months' inversion, which can affect data comparison of post-intervention assessments, which would take place in the absence of the inversion. Results of this study may raise for validity if there is concern its ิล significant discrepancy in air quality measurements.

#### Discussion

This pilot study will allow researchers to conduct future large-scale studies that implement housing strategies and interventions to contribute to safe, healthy, and energy-efficient homes. Providing participants with a summary of exposures will aid them in making necessary lifestyle changes and necessary home interventions to improve their air quality and energy efficiency. The goal of this study is to provide supporting evidence of how effective home interventions are in improving air quality and health to implement policy change. Policy changes that adopt home-interventions as necessary solutions for improving air quality and health could lead to fewer sick days, fewer health-related complications, less spending on utilities, and fewer dollars spent on ER visits, health insurance, and other health-related costs. This study offers further exploration in hopes of advocating for policy change and better air quality practices to reduce dollars spent on health care.

#### Acknowledgments

The research presented in this journal is supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under award number R25ES031497.

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### 44. Historic Traffic

### **Data and Populated**

### Weighted

### Centroids

Kevin Ramos (University of Utah); James VanDerslice (Family & Preventative Medicine, University of Utah); and Andrea Davis (University of Utah)

Faculty Mentor: James VanDerslice (Family & Preventative Medicine, University of Utah)

Pollution has been an issue in Utah since pioneers settled in the greater Salt Lake area during the mid-19th century. Articles regarding the gathering of 'smoke' in the valley tell a story of a worrisome population attempting to find solutions to this issue. Pollution measurement efforts were initiated in the early 21st century but a lack of sensors has historically created a gap in the data where assumptions must be made. This lack of resolution creates a large margin of error making it difficult to assess how different types of populations are affected by their ambiance. This study has taken into account the effects of vehicular pollution outputs as one of the main sources of exposure to pollutants. Historic traffic data was manually extracted from Utah data archives. These hand-drawn maps containing points of average traffic at a given year dating back to the 1940's were georeferenced and digitized using ArcGIS Pro. This data has the capability to interpret pollution exposures for the Utah population at a finer spatial resolution. This can give researchers a glimpse of how pollutants have impacted historic health outcomes such as cancer, blood lead levels in children, etc.

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### 45. **Reflection on**

### Undergraduate

### Research

### **Experience** - Jada

### Kali Stelmach

Jada Kali Stelmach (University of Utah)

Faculty Mentor: Hilary Coon (Psychiatry, University of Utah)

My undergraduate research experience has taught me a lot about the process of starting a study, analyzing the results, and communicating the results through writing and presenting. Before I did UROP, I did not know how to do a proper literature search or write an effective proposal. I was able to strengthen my skills in statistics, having to do many analyses and computations. I also learned more about genetics in psychiatric conditions, something that I haven't learned much about in my regular psychology and biology classes. I also had multiple opportunities outside of UROP (Undergraduate Research Opportunity Program) such as performing skin biopsies, aliquoting DNA for sequencing projects, and going to the medical examiner's office for autopsies. As I want to go into psychiatry for my career, this experience was rewarding and will prepare me for future research projects in the field.

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## 46. Characterizing the Effects of Mitochondrial DNA in Suicides

Jada Kali Stelmach (University of Utah) and Hilary Coon (Psychiatry, University of Utah)

Faculty Mentor: Hilary Coon (Psychiatry, University of Utah)

Objective: The purpose of this study was to investigate the role of mitochondrial DNA (mtDNA) in stress-related disorders that are associated with suicide risk. Identifying risk factors can help improve intervention methods and predict individuals who are at risk for suicide. Methods: mtDNA levels of 662 suicide cases were measured using whole genome sequence (WGS) data and cases were split into high vs. low mtDNA quartiles. Diagnostic status of major depressive disorder (MDD), post-traumatic stress disorder (PTSD) and anxiety were

assessed from medical records data, and polygenic risk scores (PRS) of suicides were compared between the high and low quartiles. Results: In our initial data (N = 277), results in the highest mtDNA group for the presence/absence of diagnoses of MDD (OR = 0.83; p = 0.56) and PTSD (OR = 0.72; p = 0.64) were non-significant. Anxiety was nominally significant (OR = 0.53; p = 0.05) but lost significance after adjusting for covariate effects of sex and age (p=0.08). PRS for PTSD was significantly higher in the high mtDNA group (p=0.007) adjusting for age sex, and ancestry; there were no PRS differences for MDD or anxiety. After more than doubling the sample size (N = 622), the significant associations found initially were no longer present and no further significance was observed in other characteristics. Conclusions: These results may not be as surprising as prior studies of suicide risk factors show conflicting results. It is important to replicate results as it gives us credible evidence that clinical or genetic risks are robust and generalizable across populations. However, replication can be difficult to achieve when studying the genetic and clinical risks associated with of psychiatric conditions. Non-replication in our new data suggested that our initial results likely occurred by chance. Despite the same demographics in our newer sample, we may not see a significance due to the lack of signal in the same risk factors. Future research should further examine the patterns of mtDNA to better understand the mechanism it plays in psychiatric conditions.

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### 47. Regulation of

### the Mitochondrial

### **Oxaloacetate OAC1**

Bridget Ward (University of Utah); Zachary Wilson (Biochemistry, University of Utah); and Adam L. Hughes (Biochemistry, University of Utah)

Mentor: Zachary Wilson (Biochemistry, University of Utah)

Mitochondria are organelles critical for the generation of metabolic energy in eukaryotic cells. To perform this function, mitochondria must constantly transport metabolites across the impermeable mitochondrial inner membrane. The transport of metabolites into and out of mitochondria is controlled by a family of proteins called mitochondrial metabolite carriers. These proteins are essential to the regulation of several mitochondrial processes including the citric acid cycle, ATP production, and many others. Despite their importance, little is known about how mitochondrial metabolite carriers are regulated within the mitochondrial inner membrane.

To investigate the function and regulation of mitochondrial metabolite carriers, we examined one carrier, the mitochondrial oxaloacetate carrier (Oac1), in the Baker's yeast Saccharomyces cerevisiae. We demonstrated that the Oac1 protein remains functional when fused with small epitope-tags at the N- or C-terminus. In particular, we examined the Oac1 protein in yeast strains tagged with either a Myc or HA epitope tag. By using these two different epitope tags, we unexpectedly discovered growth conditions in which the Oac1-HA protein became specifically depleted.

Upon transferring yeast cells from nutrient-rich media into media lacking specific essential nutrients, it was discovered that Oac1-HA levels decreased when switched into media lacking uracil or methionine. Similar to the nutrient-rich condition, however, Oac1-HA protein levels remained steady when the cells were moved into media lacking leucine. Conversely, we observed that the Oac1-Myc protein levels remained steady in all media conditions. A follow-up experiment was then conducted to determine whether the Oac1-HA protein was becoming stabilized in the minus leucine condition or if the protein was instead being synthesized more rapidly. To examine these possibilities, yeast strains containing Oac1-HA and Oac1-Myc were grown in nutrient-rich media then briefly incubated in either nutrient-rich media or media lacking leucine before treatment with cycloheximide, a protein translation inhibitor. We observed that Oac1-HA protein levels decreased in both media conditions after protein synthesis was arrested, while Oac1-Myc protein levels remained steady over 3 hours. This result indicated that Oac1-HA was being rapidly

degraded once protein synthesis was arrested, suggesting that the HA tag destabilizes the Oac1 protein. After discovering the media conditions in which the Oac1-HA protein became destabilized, several other HA-tagged mitochondrial proteins were examined to determine if they would provide similar results. Using truncated forms of Oac1 and tagging several other mitochondrial proteins, we showed that the HA-epitope tag generally destabilizes proteins localized within mitochondria.

The observed differences in yeast strains containing Oac1-HA versus Oac1-Myc indicate that HA-tagged Oac1 is more susceptible to being degraded when placed in certain altered metabolic growth conditions. After this discovery, we found evidence in the literature that others have seen a similar instability in HA-tagged proteins located within the cytoplasm. Future experiments will explore if the HA-epitope tag can be used to control mitochondrial protein levels if paired to a regulatable promoter. Because the HA tag appears to be inherently unstable in certain conditions, we also plan to continue experimenting with the more stable Oac1-Myc yeast strains in order to find conditions that specifically alter Oac1 protein levels.

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**DEPARTMENT IX** 

# College of Mines & Earth Sciences
# 48. Electric Bus Air

# **Quality Monitoring**

# **Platform: Findings**

#### and Implications -

### Part 1

Gabriel Baffour (University of Utah); Daniel Mendoza (Atmospheric Sciences, University of Utah; and Tabitha Benney (Political Science, University of Utah)

Faculty Mentor: Daniel Mendoza (Atmospheric Sciences, University of Utah)

In Salt Lake City, a unique air quality remote-sensing system developed and is used for air quality simulations allowing the public to view the harmful effects of regional air quality pollution in the Salt Lake Valley Region. This study focuses on integrating air quality sensors on (Utah Transit Authority) UTA and electrical buses for public transportation throughout the Salt Lake Region stretching as far south as Draper, UT, and as far north as Ogden, UT. These sensors pick up trails of Ozone, NO2, and CO2 within our troposphere. The effect these pollutants have on human health is extremely detrimental after a certain period of time, as well as induces respiratory diseases. With the aid of Utah's light rail or TRAX system, it allows for compiling data on the concentration of particulate matter with a diameter of  $<10 \,\mu m$  (PM10) around the valley region by using The Air Pollution Model. Through the use of the Air Pollution Model, forecasts can be constructed to indicate the result of different health risk patterns and pollution hot spots. Through the vast system of air quality network data, collecting and analyzing the data allows for the inquiry of recent inversions in the air caused by PM2.5 levels exceeding the National Ambient Air Quality Standard (NAAQS). In similarity, researchers have conducted a study in which a local news helicopter was in use to provide a sensor platform for spatial-temporal analysis & vertical profiles of pollution concentration in the Salt Lake Valley. In parallel, the air quality data gained by the news helicopter was disseminated from maps, websites, forecasting trends as well as public awareness. (2015-2016). Facing the increasing threat of a risk to public health through pollutants traveling through the air, it is uncertain the future of air quality, despite ensuring to limit the concentration of ozone and gas emissions.

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**DEPARTMENT X** 

# **College of Pharmacy**

# 49. Evaluations of

# **Pazopanib Efficacy**

# on Pediatric

#### Sarcoma Cell Lines

Ananya Kumar (University of Texas at Austin); Shreya Goel (Pharmaceutics & Pharmaceutical Chemistry, University of Utah); and Spencer Carstens (University of Utah)

Faculty Mentor: Shreya Goel (Pharmaceutics & Pharmaceutical Chemistry, University of Utah)

Sarcomas are cancerous tumors usually located in soft tissues and often affect pediatric patients. Despite the large amount of research surrounding cancer, there is little research pertaining to such pediatric cancers due to their rarity. Efforts

are needed to optimize current cancer treatments for pediatric settings. Pazopanib, a tyrosine kinase inhibitor, is FDA approved for various adult malignancies and is in clinical trials for pediatric sarcomas. However, it has been noted that not all patients respond to pazopanib, and many patients can develop resistance. In this project, we sought to determine the efficacy of pazopanib on two sarcoma cell lines - A-204 rhabdoid tumor and SaOS-2 osteosarcoma - using in vitro cytotoxicity assays. Cells were treated with the drug at various concentrations. Cell viability was determined using the CCK-8 assay and spectrophotometry to calculate the IC<sub>50</sub> values. The results of this assay indicate that the IC<sub>50</sub> value for A-204 cells is  $1.31 \,\mu\text{M}$ while that of SaOS-2 cells is 106.60 µM, supporting the initial hypothesis that A-204 cells are more sensitive to pazopanib than SaOS-2 cells. A clonogenic assay was also performed to determine the aggressiveness of A-204 cells in response to pazopanib in a dose-dependent manner. The assay used crystal violet staining to observe the colony formation of the cells. From the qualitative result of the image of the stained colonies, it is evident that administering increasing concentrations of pazopanib does affect the aggressiveness of the cells compared to that of the control. This data further confirms the sensitivity of A-204 cells to pazopanib. These tests lay the foundation for future in vivo studies, where imaging biomarkers will be used to identify if a tumor will respond to pazopanib and determine the onset of resistance.

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**DEPARTMENT XI** 

# **College of Science**

#### 50. Forest

### **Resilience:** Aspen

# Physiological

#### **Responses to**

# Drought

Marco Castaneda (California State University Dominguez Hills); William Anderegg (School of Biological Sciences, University of Utah); and Jaycie Fickle (University of Utah)

Faculty Mentor: William Anderegg (School of Biological Sciences, University of Utah)

Trembling aspen, P. tremuloides, plays an important role in promoting biodiversity, providing ecosystem services, and

sequestering carbon. Aspen grows in many climates and environments throughout North America, ranging from the arid conditions of the southwest to mesic conditions of more northern latitudes. Exacerbated by warming temperatures and a lack of precipitation, aspen are experiencing a range contraction caused by Sudden Aspen Decline (SAD). A garden experiment was conducted to compare how aspen from three distinct climatological regions (Dixie, Uncompanyer, and White River National Forest) will physiologically respond to one-year drought treatment. To determine which population is more likely to have a less adverse response to drought; Chlorophyll fluorescence, stomatal conductance, and leaf water potential from each individual were analyzed. There was no change in physiological responses between population and treatment groups. Which could mean that all three populations have similar phenotypic adaptations to water stress, but further research is necessary. The results could also mean that one year of treatment drought is not enough, and the experiment could express different results with repeated years of drought. Future research in tree physiological responses to drought is crucial for designing restoration projects that provide longlasting benefits for North American Forests.

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# 51. **Reflection on**

# Undergraduate

#### Research

## **Experience** - Brian

## Hassard

Brian Hassard (University of Utah)

Faculty Mentor: Orest Symko (Physics and Astronomy, University of Utah) I performed research on high-frequency traveling wave thermoacoustic engines. Building and experimenting with these devices honed my experimental skills, and led to further undergraduate research opportunities through the National Science Foundation and US Department of Energy. My undergraduate research experiences have inspired me to go to graduate school in experimental physics, where I will apply the skills I have developed to solve pressing research questions such as developing nuclear fusion reactors or optical imaging devices.

About the Author

Brian Hassard UNIVERSITY OF UTAH

52.

## Characterization

### and Analysis of a

## **High-Frequency**

# **Traveling Wave**

### Thermoacoustic

# Engine

Brian Hassard (University of Utah) and Orest Symko (Physics and Astronomy, University of Utah)

Faculty Mentor: Orest Symko (Physics and Astronomy, University of Utah)

Traveling wave thermoacoustic engines are a potentiallyhigh-efficiency method of converting heat into mechanical (or ultimately electrical) energy. A small, high-frequency engine with low onset temperatures was characterized experimentally, including its frequency spectrum and sound pressure level. The addition of a quarter-wave standing wave resonator was also investigated on a similar high-frequency engine, with inconclusive results.

The traveling wave engine was placed in an ice bath to maintain a constant temperature for the cold heat exchanger, and an electrical current was passed through a wire wrapped around the hot heat exchanger to create a temperature difference of 200  $\circ$  across a ~1 mm gap. Sound pressure measurements were taken with a pressure probe carefully inserted into the side of the engine.

The fundamental frequency for the characterized engine was 2.45 kHz. The measured frequency spectrum showed a 130 Hz full width at half maximum for the fundamental peak; in addition, the second harmonic was found to be present at 4.91 kHz, with -20 dB amplitude from the fundamental peak and a width of 80 Hz. No higher order harmonics or other frequencies were observed at more than twice the noise decibel level.

With a ~3-Watt thermal input, the maximum observed sound pressure amplitude of the characterized engine was 403 Pa, corresponding to 146 dB, a significant oscillation. Multiple experiments confirmed consistent amplitudes in the range of 300-400 Pa, depending on the quality of the engine resonance.

Finally, Peter Ceperley proposed the addition of a quarterwave resonator to looped traveling- wave thermoacoustic engines such as this [1], a practice common for larger, lowfrequency thermoacoustic engines [2]. A 3 kilohertz engine was assembled with a ~3 cm quarter-wave resonator added. The engine with the added resonator was functional, but results on the resonator performance were inconclusive due to high variability caused by difficulties in engine-resonator alignment. A maximum pressure of 585 Pa was recorded, but results fluctuated between that result and a low of 170 Pa. Further investigation is highly warranted but remained outside the scope of this project.

An additional area of future work is that of coupling multiple thermoacoustic engines into arrays for a larger potential lowonset temperature power source.

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Orest Symko UNIVERSITY OF UTAH 53. An Analysis of

**Gas-Phase Sulfide** 

### and Oxide Bonding

to Uranium by

### **Guided Ion Beam**

### **Tandem Mass**

## Spectrometry

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An important aspect of actinide chemistry is the elucidation of the nature of the interaction between the actinides and "hard" (O) versus "soft" (S) chalcogen atoms. Understanding these relationships, especially for uranium, can help to elucidate reactivities and chemical properties that can lead to more efficient and safer nuclear waste management programs. Here, U+, UO+, and US+ were prepared in the gas phase, and their kinetic energy dependent cross sections were measured using guided ion beam tandem mass spectrometry. US+ is formed through a barrierless exothermic process when the atomic metal cation reacts with CS2, with a reaction efficiency of 74 ± 15% compared to the cross section predicted by the Langevin-Gioumousis-Stevenson (LGS) model. Reactions with Xe and CO were also studied, and endothermic reactions were modeled to yield 0 K bond dissociation energies (BDEs) of 7.26  $\pm$  0.29 eV (U+-O), 5.75  $\pm$  0.13 eV (U+-S), 6.75  $\pm$  0.27 eV (SU+-O), and 5.25 ± 0.37 eV (OU+-S). These data are sufficient to characterize simple but important bonding differences between uranium and the two chalcogen atoms studied here.

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#### 54. The Emergence

#### of Chaos in Lasers

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#### **1** Introduction

Since their creation in the 1960s, lasers have become valuable tools not only in the scientific com- munity but in daily life (laser pointers, barcode scanners, printers, communication systems, etc.). Most lasers are operated in what is known as the lasing regime, where the emitted light is co- herent, meaning it has well defined phase, amplitude, and wavelength. Additionally, lasers can operate chaotically. This chaotic regime is less commonly used but is actively researched and has applications in random number generation, communications, and metrology [5].

Chaos is well known to only appear in systems that are described by a system of differential equations with a minimum of three dimensions. In some regimes, the behavior of a laser can be modelled with a two (or even one) dimensional system of equations [1]. More complicated and precise models increase the number of dimensions of the system of equations and thus can describe chaotic laser operation [1] [2]. This paper will compare a two dimensional model of a laser to a three dimensional model and explore the emergence of chaos in the three dimensional model.

#### 2 Models

The two models presented here both describe lasers but focus on different characteristics of the laser. Additionally, we will be focusing on three regimes lasers can operate in: non-lasing, lasing, and chaotic. Neither of these models capture the full complexity of laser operation, and examples of more intricate laser operation abound [3] [4] [5]. However, these simulations do serve to demonstrate the emergence of chaotic behavior even in greatly simplified models of lasers.

#### 2.1 Two-Dimensional

The two-dimensional system of equations is presented in [1]. It is a coupled two-dimensional system of ordinary differential equations, given by eqs. (1) and (2).

$$n' = GnN - kn (1)$$
$$N' = -GnN - fN + p (2)$$

In this model, n represents photon number, N represents the number of excited atoms, n<sup> $\cdot$ </sup> and N<sup> $\cdot$ </sup> represent the time derivatives of n and N, G is the gain of the laser, k is the loss rate of photons, f is the decay rate of the excited state, and p is the strength of the pump. Since the focus of this paper is analyzing the behavior of the solutions of these equations, we will rewrite (1) and (2) in a simpler dimensionless form via the substitutions x=Gn/k, y=GN/k, a=f/k,  $\tau$ =kt, and b=Gp/k<sup>2</sup> [1].

Now, (1) and (2) are rewritten as (3) and (4).

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As mentioned earlier, chaos does not appear in this model since it is two-dimensional. However, it can describe the nonlasing and lasing regimes. Here, the transition from non-lasing to lasing occurs when b becomes larger than a. Once this occurs, x, the dimensionless equivalent to photon number, will approach b–a as time goes to infinity. The transition from nonlasing to lasing is shown in fig. 1, where (3) and (4) were numerically integrated using a fifth order Runge-Kutta method in Python to generate the plots. In all situations, x and y were initially set to 1. Fig. 1(a) shows results from a simulation which used a = 1.5, b = 1.0, 1(b) shows results with a = 1.0, b = 1.0, and 1(c) shows results with a = 1.0, b = 1.5.



Fig. 1 clearly shows x going to zero below the transition, slowly

going to zero at the transition point, and approaching the expected finite value above the transition point.

#### 2.2 Maxwell-Bloch Equations

The three-dimensional equations studied here are known as the Maxwell-Bloch equations. Eqs. (5)- (7) show the dimensionless form, as presented in [1] and [2]. These equations were also numerically integrated using fifth order Runge-Kutta method in Python to generate the plots shown later in this section.

$$E^{\cdot} = \kappa(P - E) (5)$$
  
 $P^{\cdot} = \gamma_1(ED - P) (6)$   
 $D^{\cdot} = \gamma_2(\lambda + 1 - D - \lambda EP) (7)$ 

This model treats the laser semi-classically and thus models the electric field, E, instead of the photon number, n. The other two quantities, P and D, represent the mean polarization of the atoms and the population inversion, respectively. Electric field and polarization are, in general, vector valued. Here we assume that they always point in one direction and thus E and P represent the magnitude of these vectors in that direction.  $\kappa$  corresponds to the decay rate from light transmission,  $\gamma 1$  corresponds to the rate of depolarization,  $\gamma 2$  is the population inversion decay rate, and  $\lambda$  is related to the pump strength. Here,  $\lambda < 0$  corresponds to a pump which is not strong enough to sustain population inversion, the collective excitation of the lasing medium.

The non-lasing regime, where E tends towards zero, is thus defined by setting  $\lambda < 0$ . This roughly corresponds to the situation where the strength of the pump is too low to force lasing behavior and either incoherent emission, like a lamp, or no light emission at all, is the result. Fig. 2 shows electric field E in this regime, where initially E = P = D = 0.5,  $\kappa = \gamma 1 = \gamma 2 = 1$ , and  $\lambda = -1$ . Fig. 2(b) shows that the trajectory

quickly approaches the origin, hence no coherent electric field is produced and lasing does not occur.



#### Figure 2

The lasing regime is defined by 0  $\leq$   $\lambda$  <  $\lambda_{H},$  where  $\lambda H$  =  $\kappa$ 

 $\left(\frac{\frac{\kappa}{\gamma_1} + \frac{\gamma_2}{\gamma_1} + 3}{\kappa - \gamma_2 - \gamma_1}\right)$  -1 so long as  $\kappa > \gamma 2 + \gamma 1$  In this regime, E will exhibit damped oscillations around 1 or -1. This stable equilibrium corresponds to a coherent, polarized electric field and hence proper lasing. Fig. 3 shows a situation whereEoscillatesaround1,withE=P=D=0.5initiallya nd $\kappa = \gamma 1 = \gamma 2 = \lambda = 1$ . Additionally, the time length was extended to better capture the behavior.



#### Figure 3

The Maxwell-Bloch equations are capable of demonstrating a third chaotic regime which the two-dimensional equations were unable to predict. This regime is defined by  $\lambda > \lambda H$  and  $\kappa > \gamma 2+\gamma 1$ . Once this condition is fulfilled, the system undergoes a subcritical Hopf bifurcation. As shown in fig. 4, this regime has E aperiodically switching between oscillations around 1 and -1. This figure uses E=P =D=0.5 initially along with  $\gamma 1 = \gamma 2 = 1$ ,  $\kappa = 3$ , and  $\lambda = 21$ .



#### Figure 4

In order to demonstrate the sensitive dependence on initial conditions, fig. 5 shows the results of a simulation which used the same parameters as fig. 4, but with E now set to 0.51.



A comparison of figs. 4 and 5 clearly demonstrates sensitive dependence on initial conditions, as expected in a chaotic system. The final points are at different positions in phase space and the timing of flips between 1 and -1 become completely uncorrelated.

What this theory physically predicts is an operating regime in which a laser emits monochromatic light which chaotically fluctuates in intensity and switches polarization. In actuality, this quite hard to realize experimentally. is Most demonstrations of chaos in lasers arise in more complicated situations which this model does not accurately describe [5]. As shown in [3], more complicated laser systems show more than three operating regimes and show different types of chaotic behavior. Chaotic behavior similar to that predicted by the model described here is demonstrated experimentally in [4], which was published decades after the theoretical prediction of this sort of chaos.

The strange attractor that shows up in the chaotic regime is remarkably similar to the famous Lorenz butterfly, a strange attractor which arises from the Lorenz system given in eqs. (8)-(10). In

fact, as shown in [1] and [2], the transformation  $t \rightarrow (\sigma/\kappa) t'$ ,

$$E \to \alpha x, P \to \alpha y, D \to (r - z), \gamma 1 \to k/\sigma, \gamma 2 \to (\kappa \beta)/\sigma$$
, and  
 $\alpha = \frac{1}{\sqrt{b(r-1)}}$  exactly transforms the

 $\lambda \rightarrow r - 1$ , where **V** (1 - 1), exactly transforms the Maxwell-Bloch equation into the Lorenz system.

$$\dot{x} = \sigma(y - x)$$
 (8)  
 $\dot{y} = rx - y - xz$  (9)  
 $\dot{z} = xy - bz$  (10)

#### 3 Conclusion

The modelling of lasers leads to sets of equations which work as wonderful examples of the emergence of chaos. Both models here were able to describe the primary lasing and non-lasing regimes of laser operation, but the simpler model left out key details which allow for the exploration of a chaotic laser. This serves as an example of the way new behaviors can be revealed through more detailed modelling and simulation.

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#### 55. Relating

### **Student's Comfort**

#### Level with

#### **Classmates to**

#### Social Belonging in

#### **General Chemistry**

#### 1

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This study focuses on the factors that students use to describe their social belonging in General Chemistry 1, specifically the factors they use to describe their level of comfort with their peers in the classroom. Previous studies in the Frey group have shown that students' social belonging

affects their grades and persistence in the general chemistry series and introductory physics 1 course at the University of Utah. These studies have also identified two main components for social belonging: sense of social belonging and the belonging uncertainty. A sense of social belonging is the sensitivity of an individual's connectedness with people such as peers and instructors and the significance of the course environment that helps build an interpersonal relationship of how they feel they fit in the course. One aspect of courselevel sense of belonging is the level of comfort that students have with their classmates. While these recent quantitative studies show that social belonging affects student performance and retention in introductory STEM courses, there are very few studies that ask students what factors they look at when describing their belonging in STEM courses and none in these introductory STEM courses. In this current study, student responses from General Chemistry 1 about their comfort level with their peers were analyzed to generate a codebook that contains eight remote and non-remote categories: Course Environment, Common Academic Experience, Identity, Perceived Ability, Student-Student Relationship/Interaction, Student-Instructor Relationship, Non-Specific, and Non Codable. In this presentation, I will present these categories, their definitions, and representative quotes for each category. I will also discuss implications for instructors to improve the inclusivity of their STEM courses, and the next steps for this project.

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# 56. Interpreting the

# **Consequences of**

# Single-Cell CRISPR

## Perturbations

# **Through Label**

## Transfer

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Single-cell RNA sequencing (scRNA-seq) permits the measurement of gene expression at single cell resolution, enabling a new understanding of the cell and molecular responses to perturbations in developing animals. However, a challenge for using scRNA-seq data exists in defining shared cell types across samples. My objective is to develop an agnostic computational framework to transfer cell type labels onto a perturbed sample and analyze how gene expression and cell type abundances are affected. To test this label transfer method, I focused on the gene regulatory network that establishes mesoderm cell types in the zebrafish embryo. The genes of interest in this network, noto, tbxta, and tbx16, are wellknown mesoderm regulators, but have not been studied at single-cell resolution. Using CRISPR, I perturbed each transcription factor and generated scRNA-seq libraries for each mutant at 24hpf. Each condition's gene expression profiles were processed and integrated through Seurat's clustering workflow to aggregate the cell populations present in all conditions. Marker genes for each cluster of the integrated object, with all perturbed conditions, act as the input for the annotation framework to identify the most likely cell type for each cluster. By employing this framework, I identified the different cell types present across each perturbation condition in the developing embryos. Preliminary analysis of the labeled singlecell datasets suggests changes in cell abundance of cell types previously known, such as changes in notochord and muscle. Further analysis and experiments are required to validate the cell type abundance changes seen in these datasets. The annotation framework has allowed me to begin studying

mesoderm patterning interactions between *noto*, *tbxta*, and *tbx16* at a molecular level. Due to the agnostic nature of this computational framework, this method is broadly useful for studying cell and gene expression in other single-cell perturbations.

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to Detect

## Functional

## Interactions

## Important for the

# **DNA Repair**

# **Enzyme MUTYH**

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#### Abstract

DNA is the blueprint of all biological life as it provides the means necessary for sharing of genetic information. DNA is formed and stabilized by interactions between four unique bases, Adenine (A), Cytosine (C), Guanine (G), and Thymine (T) which come together in predetermined patterns to create a two-stranded structure known as the double helix. Genetic information is stored in the precise pairing and arrangements of the four bases, encoded in the form of a sequence to allow correct information to be sent to replicated strands.

Adenine must pair with Thymine, and Guanine must pair with Cytosine to retain the stability and information of the DNA. However, exposure of DNA to reactive oxygen and nitrogen species (ROS) lead to chemical damage, DNA base modifications, and incorrect pairings of bases. This mispairing caused by DNA damage can ultimately result in mutations, permanent changes to the information encoded in the DNA sequence, which can lead to malignant cellular phenotypes and life-threatening diseases such as cancer (Banda et al. 2017).

For billions of years, biological systems have been exposed to DNA damage, creating selective pressure that resulted in an ensemble of DNA repair strategies through evolution. My Honors Thesis focuses on the base excision DNA repair enzyme MutY in bacteria and its homologous protein in humans, MUTYH. MutY and MUTYH prevent the accumulation of mutation in DNA by working with partner proteins belonging to the GO DNA repair pathway.

MutY and MUTYH prevent the accumulation of mutations by recognizing and initiating repair at sites where 8-oxo-Guanine mispairs with Adenine (8OG:A mispairs). The 8OG:A mismatch arises due to oxidation of Guanine in G:C pairs followed by replication, and if not repaired can lead to permanent G:C to T:A mutations. In humans, these mutations may lead further to lung, rectal, and colon cancers. The GO pathway involves the recognition, excision, and replacement of the mismatched 8OG:A base pair to restore the authentic G:C base pair. MutY initiates repair on the 8OG:A lesion, and repair is completed with the help of other GO pathway proteins XthA and Nfo which work with MutY in bacteria, as well as APEX1 and OGG1 which work with MUTYH in humans. The GO DNA repair pathway is very complex, and it is not clearly understood how MutY and MUTYH are interacting with their partner proteins. Understanding the functional and physical interactions of these proteins with each other would provide crucial insights and allow us to make predictions about their behaviors and reactions in medical and pharmaceutical settings.

It is known that MutY and MUTYH depend on other proteins, but the exact mechanism of their interaction is still unclear. In my Honors Research I applied Bioinformatics to look for evidence of functional interactions among GO partner proteins as recorded in the evolutionary history of these proteins. A statistical calculator known as Mutual Information (MI) measured pairwise codependency and associations between specific residues belonging to MutY or between one residue belonging to MutY and another residue belonging to a GO repair pathway partner protein. The analysis requires finding many examples of the protein from several different species of organisms. Fortunately, most organisms have a MutY or MUTYH encoding gene. In one set of experiments I looked for functional interactions between residues across the N- terminal and C-terminal domains of MutY and MUTYH, and examined how the results changed as the number of species increased from 50 species to 5000 species. In a second set of experiments I looked for functional interactions between residues belonging to MutY and its GO repair partner proteins, XthA and Nfo. In a final set of experiments I looked for functional interactions between MUTYH and its GO repair proteins, OGG1 and APEX1.

Through several experiments with the MI calculator I have found evidence for functional interactions between MutY and its partner proteins, between MUTYH and its partner proteins, as well as between the two domains of MutY and between the two domains of MUTYH. The number of species included for MI analysis had a big impact in raising the confidence level, such that experiments with more than 200 species gave the best results with confident evidence supporting coevolution and functional interactions.

To further interpret the functional interactions indicated by high MI scores, I visualized the functionally significant residues in a three-dimensional molecular environment with help from the molecular display program ChimeraX. The interactions identified by Bioinformatics and visualized at the molecular level suggest that proteins are evolving together. By observing the residues that have been deemed as important, functional, and evolutionary significant in this thesis, it can become possible to create better mechanism maps of how MutY and MUTYH work, how they interact with their partner proteins, and if certain regions in the protein can be targeted by medicine to reduce the development of cancer or increase the chances of curing cancer in patients with existing lung, rectal, and colon cancers.

### Introduction

Although researchers have been able to understand

the structure and role of Deoxyribonucleic Acid (DNA) for several decades (Watson and Crick 1953), it has only recently been possible to access large databases containing sequences of DNA encoding proteins, enzymes, and functional RNAs. In 1944, Avery, MacLoad and McCarty showed that the uptake of pure DNA from a virulent bacterial strain could confer virulence to a nonvirulent strain (Avery, Macleod, and McCarty 1944). Their work was followed by Hershey and Chase validating the role of DNA as a genetic information encoding molecule (Hershey and Chase 1952), and by Watson, Crick and Franklin finally explaining the double-helix structure of DNA in 1953 (Watson and Crick 1953). Following that, it took approximately 20 or more years of technological advances before the first DNA sequencing method became available for use (Sanger, Nicklen, and Coulson 1977; Maxam and Gilbert 1977). In the 1990s, large databases established efficient search tools such as the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI). In 2002, a database for sequences called GeneBank began including whole genome shotgun sequences generated by a semi-automatic technique (K. Smith 2013; K. A. Smith 2008). This recent easy access to large data sets and increasing availability of user-friendly search tools has enabled researchers to ask questions about the function and evolution of proteins across species

of organisms, a study that falls into the field called Bioinformatics. Bioinformatics allows us to find for the functional and physical evidence interactions between proteins, domains of proteins, and amino acids, that have worked together through billions of years at a molecular level to improve certain functions such as prevention of mutations in DNA. My Honors Thesis applied a approach to understand Bioinformatics the particular system associated with MutY and MUTYH DNA repair proteins that is dedicated to prevention of mutations and called the GO DNA Repair pathway to detect Mutations in DNA resulting from DNA damage. Exposure to reactive oxygen and nitrogen species (ROS) damages the chemical structure of DNA, adding or changing the bases, and breaking the on atoms phosphodiester backbone. These DNA damaging lesions are especially dangerous during DNA replication because the altered chemical structures can now become permanent mutations caused by incorrect pairing of base pairs (Banda et al. 2017). of these mispairs known the One as 8-oxo-7,8-dihydroguanine: Adenine (80G:A) mismatch is caused due to the low redox potential of guanine, meaning a high susceptibility to oxidation, coupled with template ambiguity since 80G can pair with either C or A in the active site of DNA polymerase during DNA replication (Yeang and Haussler 2007; Trasviña-Arenas et al. 2021).

MutM (Fpg), MutY, and MutT in bacteria and their functional counterparts in humans OGG1, MUTYH, and NUDT1 respectively, act as a three-part defense system to protect against G:C to T:A mutations. This defense system is called the GO DNA repair pathway and relies on base excision to remove adenine from 8OG:A mismatches as the first step in restoring the authentic G:C base pair (Figure 1).

In the GO repair pathway in Eukaryotes, OGG1 recognizes and excises the damaged 80G from the lesion leaving behind an 8OG:C Apurinic/ apyrimidinic (AP) site, meaning a deoxyribose sugar with no base attached, also called an abasic site. Adenine excision by OGG1 is followed by APEX1 which hydrolyzes the phosphodiester DNA backbone at the AP site, thus making a substrate for DNA polymerase to fill in the missing C nucleotide and DNA Ligase to seal any nicks in the DNA. If DNA replication occurs before OGG1 can find and initiate repair at the OG:C lesion, there is a chance that one of the daughter DNA molecules will now have an OG:A lesion since OG can template insertion of C through Watson-Crick-Franklin base pairing or template insertion of A through a Hoogsten base pair. One more round of replication propels the OG:A lesion into a permanent T:A mutation. To avoid this outcome, MUTYH is dedicated to finding and initiating repair at the OG:A lesion by excising the adenine base. Similar to repair at the OG:C lesion, the resulting AP site

across from OG is processed by all of the same GO repair pathway partner proteins APEX1, DNA polymerase, and DNA ligase, to restore an OG:C base pair, giving OGG1 a second chance to complete the repair process.

It is important to note that OGG1 and MUTYH have similar functionalities in that OGG1 removes OG from OG:C base pairs and MUTYH removes A from OG:A base pairs. However, there are instances where the two have to work parallel to each other if OGG1 is unable to recognize or excise the lesion in DNA (Banda et al. 2017)). The exact mechanism of how MUTYH and OGG1 collaborate is still unclear. While we know a lot about the mechanism of OGG1 and MUTYH acting on DNA to repair and prevent mutations, the exact mechanism and interactions between the GO DNA repair pathway proteins is relatively underexplored. For instance, how do such proteins work together to coordinate repair at OG:C and OG:A lesions? And can their physical interactions and communications allow us to create better mechanism maps of their repair pathway?

Similar questions apply to the GO Repair Pathway in the Eubacteria domain where MutY interacts with its two partner proteins, Nfo, and XthA to recognize, excise adenine and replace the mismatched 8OG:A with 8OG:C, restoring a substrate for the MutM-encoded enzyme Fpg which functions analogously to OGG1 in mammals. As observed in Figure 1, communication between multiple proteins is essential in this complex GO DNA Repair pathway. However, exactly how these proteins communicate with each other is still unclear and understudied.

At the intramolecular level, N-terminal and C-terminal domains play critical roles in the function, activity, folding, stability, and shape of MutY and MUTYH. These two domains work together and provide distinct functions to find and initiate repair at 80G:A mispairs (Bhardwaj et al. 2010). For instance, the N-terminal domain (NTD) in MutY exhibits an adenine-specific binding pocket and contains the catalytic residues that are required for base excision (Woods et al. 2016), whereas the C-terminal domain (CTD) plays an important role in 8OG recognition (Russelburg et al. 2019). Communication between the CTD and the NTD is crucial since removing Adenine at other locations in the DNA would accelerate mutations instead of preventing mutations. While we recognize the separable biochemical functions for the NTD and CTD, we still do not understand how the two domains "talk" to each other to relay the information that yes, this is an authentic 80G:A lesion, and it is "OK" to remove the adenine here. To understand this question and uncover the functional interactions responsible for this relaying of information, I applied Bioinformatics in my Honors Research project.

Bioinformatics allows for a statistical measure of codependency between two proteins or domains of proteins, also called Mutual Information (MI, see Figure 2), making it possible to find evidence for functional interactions between two different proteins or between two domains in the same protein. Bioinformatics and MI enabled this thesis to draw conclusions and create assumptions behind the precise molecular team work and behavior of the proteins involved in the GO DNA repair pathway. My Honors Thesis will demonstrate that traceable patterns of co- changing regions exist in the sequence alignments of proteins belonging to the GO DNA Repair pathway as detected by statistically significant hig MI scores.



Figure 1. The GO DNA repair pathway. A: In bacteria, MutY acts as a final line of defense to prevent G:C to T:A mutations. Figure from Russelburg et al., 2020. B: In mammals, the GO repair pathway features the key base excision repair enzymes OGG1 and MUTYH. Figure from Banda et al., 2017).

$$\sum\limits_{j=1}^n \sum\limits_{k=1}^m p_{jk} \log rac{p_{jk}}{p_j q_k}$$

Figure 2. Mutual Information calculation. The Mutual

Information (MI) calculation detects interesting covariation between two sets of data defined by j elements for one data set and by k elements in the second data set. For my application, the j elements are the different amino acid residues at one particular position in the sequence alignment for Protein A, and the k elements are the different amino acid residues at a second particular position in the alignment of Protein B. The variables pj and qk refer to the frequency with which the different k and j amino acid residues are observed at the two positions. Importantly, pjk is the frequency observed for the combination of a pair of amino acids at the two positions. In some cases, Protein A and Protein B are two domains from the same protein, as is the case when looking for covariation for positions in the NTD and CTD of MUTYH, for example. The calculation is repeated for all possible pairwise position comparisons in the two proteins.

#### Results

DNA replication involves a tradeoff between speed and accuracy meaning mispairing of the nucleotides on the template and newly synthesized strand is inevitable. MutY in bacteria and MUTYH in eukarya play important roles in prevention of mutation that otherwise would result from 8-oxo-7,8-dihydro-guanine:Adenine (8OG:A) misspairs and work with other proteins in the GO DNA repair pathway to restore the authentic G:C base pair. If left untreated and unrecognized, the pile up of G:C to T:A mutations can lead to lung, rectal and colon cancers as is the case for certain heritable alleles of MUTYH which underperform and predispose family members to early onset disease. In order to understand the teamwork and mechanistic relationships between MutY and MUTYH and their partner proteins in the GO DNA repair pathway, it is important to observe their interactions. The working hypothesis of my Honors Thesis research is that evidence for functional interactions involving MutY in bacteria or MUTYH in eukarya is recorded in the evolutionary history of these proteins. Taking a Bioinformatics approach allowed me to detect covariation over millennia of evolution, measured in the Mutual Information (MI) score and infer functional interactions between two proteins or two domains of the same protein. To calculate MI score, I used the Mutual Information (MI) Calculator which was previously written in the R programming language by Dr. Martin Horvath from the School of Biological Sciences, College of Science, at the University of Utah. A detailed description of how to run the MI calculator can be found in Methods. Essentially, the MI calculator examines alignments of protein sequences to measure the codependency between two amino acid positions (columns) in those sequence alignments, always pairing a column from protein A with a column from protein B. Covariation is expected over the course of evolution if the two amino acid positions are paying attention to each other through functional interactions. The results are summarized into a single graph called the MI distribution graph.

For the calculations to work properly, it is essential to follow the workflow outlined in Figure 3. All experiments required sequences to be downloaded as FASTA files from the correct database and to be put into PROMALS3D to create an alignment of the sequences. The alignments were adjusted by adding the correct headers and removing extra gaps as calculated by R programs I created using the R Studio interactive developer environment (IDE). Protein alignments were analyzed by the MI calculator, and interesting positions in the proteins identified by especially high mutual information scores were visualized in molecular models created with the program ChimeraX.

### **Interpreting the MI Score Distribution**

As we will be looking at several outcomes from different experiments, it is useful to begin with how to interpret the MI score distribution. The MI calculator works by measuring covariation in alignments of amino acid sequences from two separate proteins, reporting the level of covariation as the Mutual Information (MI) score for each possible pairwise comparison of positions in Protein A and Protein B. These MI scores are plotted as distribution labeled "Info", а an abbreviation of Information, with purple bars as seen in Figure 4. MI score is indicated along the Xaxis of the graph, and as we move to the right of the graph, the MI score increases. High MI scores are

the interesting outcomes reflecting a high degree of covariation and are taken as evidence for functional interaction.

However, from the purple bars alone we cannot conclude which mutual interactions are statistically significant. Accordingly, we tested for statistical significance by randomizing the species order and repeating the MI calculation to produce the Random distribution labeled "Rand", as shown with the blue bars in Figure 4. The Random distribution defines regions of the MI score that are caused by random chance and are not statistically significant. The degree to which the two distributions, Information and Random are different, especially in the right-hand portion of the graph reporting high MI scores, gives us confidence that the high MI scores are statistically significant. Furthermore, if the two distributions are highly similar, there is possibly no detectable covariation between the proteins, or data have not been handled correctly while conducting the experiment.

## Validating the MI Calculator Results

Given the unfamiliar nature of the MI Calculator in looking for patterns of covariation, it was necessary to check its performance before conducting more experiments. Specifically, I wanted to be sure that the test for statistical significance was working. For example, would the statistical significance test alert me if there are no functional interactions between Protein A and Protein B? I was concerned about MI score validity because initial tests almost always gave interesting patterns with strong differences between the Information and Random distributions. What would the results look like if the two proteins have no chance to interact?

To answer these questions, an experiment was conducted in which the N-terminal domain (NTD) of MUTYH was compared to the NTD of MutY. In this situation, there is no possible way for the two domains to interact since one is evolving in mammals and the other is evolving in bacteria. This experiment was conducted two times using different numbers of species for verification. Since MUTYH and MutY are from different domains of life, Eukarya and Bacteria respectively, the MI distribution graph should show no functional interactions, such that all mutual interactions can be explained by random chance, if the MI calculator and the quality control check were working correctly. As seen in Figure 5, all mutual interactions between the two domains reported in the Information distribution (Info, purple) were equal in value and height with the Randomized interactions (Rand, blue). These MI scores and MI distribution graphs explain that no functional interactions which were not rejected by our control experiment were observed between the two domains of life which had separately evolved from each other billions of years ago. This experiment

therefore validates the strategy for statistical significance, giving me confidence that the MI Calculator works properly and accurately.



Figure 4. MI Distribution Graph. The example is taken from the analysis of MutY NTD versus MutY CTD across 50 species. A,B: Separate graphs produced for analysis when species are paired (A) and when species order is scrambled (B). C: Combination of the Information and Random distributions to produce the MI

distribution graph. The Information MI score distribution (Info, purple) extends to higher MI scores plotted along the X-axis compared to the Random distribution (Rand, cyan), indicating that not all mutual interactions are by random chance. The difference between the Info and Rand MI scores in the righthand region of the plot reporting rare, high-scoring positions indicates statistically significant interactions between the two domains of MutY.



Figure 5. Checking Mutual Interactions between the NTD of MutY and NTD of MUTYH. Graphs A looks at interactions between 78 species of MUTYH NTD and 50 species of MutY

NTD. Graph B looks at the interactions across 50 species MUTYH NTD and 50 species MutY NTD. Random interactions (Rand, purple) match or surpass the value of Information interactions (Info, cyan) in both graphs, indicating that all detected interactions between the two domains are by chance and not statistically significant. This important result validates the use of the Random interaction distribution as a test for statistical significance, demonstrating that it works for both large and small numbers of species.

# Choosing Between UniProt and NCBI Databases

UniProt, a freely accessible database of protein sequences, was initially used to obtain fasta sequence files of MUTYH and MutY proteins from 50-100 species . In attempting to increase the number of species included, I was faced with limitations on the UniProt database where selecting more than 200 species became difficult. I aimed to look at co-changing patterns between proteins across 500 and 5000 species to increase the confidence interval of the MI scores. Furthermore, my ability to clean the sequences obtained from UniProt relied on the TCSH Command Line tool, which further limited performance and reduced time efficiency in conducting the experiments. To speed up the process time as well as gain access to larger samples of sequences and species, I explored a new sequence database, the National Center for Biotechnology Information (NCBI), which is part of the United States National Library of Medicine (K. Smith 2013). Different from UniProt, with NCBI I

could apply the Basic Local Alignment Search Tool for Proteins (BLASTP) to quickly retrieve hundreds and thousands of sequences using a single fasta or accession ID of the desired protein as an input (K. Smith 2013).

While NCBI provides easier, more efficient access to a large number of sequences, it includes isoform sequences, reducing the quality of data compared to that sourced fromUniProt, which does a better job of filtering isoforms and focusing on unique species. This dilemma of choosing between quantity versus quality for NCBI and UniProt, respectively, was resolved by comparing MI results from three separate experiments as seen in Figure 6. Protein sequences for these three experiments were obtained using the two databases, NCBI in panel A, and UniProt in panel B. The three compared results experiments of mutual interaction between MUTYH versus OGG1 at 50 species, MutY NTD versus CTD at 500 species, and MUTYH NTD versus CTD at 500 species level. At both 50 and 500 species UniProt returned better by producing graphs results with more distinguishable differences between MI scores (Info) and the control scores (Rand) obtained after randomizing the order of the species for Protein A and Protein B. The degree to which the MI score distribution (Info) extends past the control scores (Rand) gives us greater confidence that the very highest MI scores are statistically significant and report important functional interactions, and this was the case for the sequences sourced from UniProt. Notably, NCBI performed almost identically to UniProt for 500 species in the MUTYH NTD versus MUTYH CTD experiment, but performed poorly in the MutY NTD versus CTD experiment with 500 species and also poorly in the OGG1 versus MUTYH experiment with 50 species. This comparison analysis concluded that NCBI should be reserved for experiments involving more than 500 species, and UniProt can provide an initial source of the sequences needed for Bioinformatics and performs better than NCBI if analyzing 500 or fewer sequences.

In retrospect these results were perhaps expected as UniProt uses a Knowledge Base tool known as UniProtKB finds high quality UniProtKB. sequences extracted from the literature and curator-evaluated computational analysis, where novel function, structural and biochemical data have been assigned priority (UniProt Consortium 2007). NCBI, on the other hand, uses the Basic Alignment Search Tool (BLAST) with little or no intervention. BLAST human uses UniProtKB\_RefSeq mapping provided by the UniProt Consortium to retrieve sequences that are 100% identical to the RefSeq protein and are either from the same organism or use the same protein accession numbers. NCBI also computes related sequences which can include UniProtKB sequences

to be displayed in a separate section in the results page of the BLAST Tool (Wheeler and Bhagwat 2007). NCBI also uses the algorithm of returning sequences which are almost identical to the parent protein, in small numbers of sequences, which can greatly reduce our chances of getting any diversity in our pick of species and therefore will reduce the number of functional interactions that can be calculated by MI Calculator. This is harmful to my downstream application because diversity in sequences helps MI with calculation of covariation, however if diversity is reduced by NCBI's algorithm, MI will not produce good and accurate results in experiments involving a small number of species. With these considerations in mind and with the goal of obtaining better data, sequences for 500 or fewer species were obtained using UniProt, whereas NCBI provided sequences for the larger experiments with more than 500 species.

# Mutual Information Depends on the Number of Species

In the initial steps of my experiment using the two domains of N-terminal and C-terminal domains as my two proteins in the MI calculator, I included a small number of species to quickly detect functional interactions. I wondered if these patterns would be strengthened by including more species to capture a larger cross-section of evolutionary history. To test how Mutual Information behaved as a function of the number of species included I repeated a few experiments with different numbers of species ranging from 100 to 5000 species.

The two proteins were tested across three different numbers of species, 50 (100 for MUTHY), 500, and 5000. To highlight differences in the MI score distributions as a function of the number of species, I estimated a Z-score, the number of standard deviations that is below or above a reference point such as the mean value of measurement. The Z-score for an MI distribution was calculated by taking the difference in maximum MI score observed for the Information distribution and the Random distribution, and dividing that difference in maximum scores by the MI cutoff value defined by the maximum score in the Random distribution. The point at which Random distribution stops in the MI distribution graph is referred to as MI Cutoff in Table 1. Since Z-score is an estimation of the confidence interval in statistics, as Z-score increases, my confidence in MI scores for the Information distribution increases.

As is seen in Figure 7 and Figure 8, both MUTYH and MutY showed an increase in the number of functional interactions detected between the NTD and CTD domains as the number of species increased, increasing the confidence of data as indicated by Z-score. It is of note that the value of MI cutoff decreased almost linearly as the number of species increased. This behavior showed that as the number of species increases, the number of functional interactions will also increase which in turn will increase the confidence interval in MI score as shown by Z-score.



Figure 6. Comparing NCBI and UniProt. A: Left-hand panel of the figure shows outcomes for sequences extracted by NCBI. B: Right-hand panel shows outcomes for sequences extracted by UniProtKB. See text for further interpretation. Table 1: Summary of Results

| Proteins<br>analyzed   | Target<br>number<br>of<br>species | Database | Number of<br>statistically<br>sig. MI<br>Score | MI<br>Cut<br>off | Z-<br>score | Interacti<br>on |
|------------------------|-----------------------------------|----------|--|------------------|-------------|-----------------|
| MUTYH<br>NTD vs<br>CTD | 100                               | UniPort  | 83   | 0.8              | 1.3         | YES             |
|                        | 500                               | UniPort  | 308  | 0.4              | 2.4         | YES             |
|                        | 5000                              | NCBI     | 2565   | 0.15             | 4.3         | YES             |
| MutY<br>NTD vs<br>CTD  | 50                                | UniPort  | 49   | 1.0              | 0.9         | YES             |
|                        | 500                               | UniPort  | 384  | 0.5              | 1.5         | YES             |
|                        | 5000                              | NCBI     | 2546   | 0.15             | 2.0         | YES             |
| MUTYH<br>vs<br>OGG1    | 50                                | UniPort  | 13   | 1.0              | 0.2         | NO              |
|                        | 100                               | UniPort  | 20   | 1.1              | 0.1         | NO              |
|                        | 250                               | UniPort  | 34   | 0.7              | 0.2         | ERHAPS          |
| MUTYH<br>vs<br>APEX1   | 50                                | UniPort  | 13   | 0.78             | 0.1         | YES             |
|                        | 100                               | UniPort  | 30   | 0.6              | 0.6         | YES             |
|                        | 250                               | UniPort  | 12   | 0.5              | 0.6         | YES             |
| MutY vs<br>XthA        | 50                                | UniPort  | 3  | ND               | ND          | NO              |
|                        | 100                               | UniPort  | 16   | ND               | ND          | NO              |
|                        | 250                               | UniPort  | 29   | ND               | ND          | NO              |
| MutY vs<br>Nfo         | 50                                | UniPort  | 3  | ND               | ND          | NO              |
|                        | 100                               | UniPort  | 11   | 1.63             | 0.1         | YES             |
|                        | 250                               | UniPort  | 25   | 1.25             | 0.3         | YES             |



Figure 7. MI Distribution graphs between the NTD and CTD of MUTYH. Graphs A to C: Looking at mutual interactions between the two domains across 100 to 5000 species. Graph D: Linear and positively correlated relationship between number of species and Z-score. As the number of species increases, confidence in MI also increases.



Figure 8. MI Distribution graphs between the NTD and CTD of MutY. Graphs A to C: Looking at mutual interactions between the two domains across 50 to 5000 species. Graph D: Linear and

positively correlated relationship between number of species and ZZ-score. As the number of species increases, confidence in MI also increases.

Partner Protein Interactions for MutY and MUTYH

Having established that N-terminal and C-terminal domains within MutY and within MUTYH are coevolving, I next applied the MI calculator looking for functional interaction between DNA repair proteins in the GO Repair Pathway. Specifically, I looked for evidence that MUTYH is functionally interacting with OGG1 and, separately, if MUTYH is interacting with APEX1. As MUTYH and OGG1 have to work in sync with each other to recognize OG:A mispairs and remove the Adenine, and at a later stage the OG, I predicted that the two proteins would have functional interactions with each other as detected in covariation reported by the MI Calculator. As seen in Figure 9, evidence for interactions between MUTYH and OGG1 may exist, however it is very weak, as inferred from low Z-scores regardless of the number of species analyzed. The low Z-score between OGG1 and MUTYH might indicate that proteins are not functionally interacting with each other, or an issue existed with the sequence alignments. The problem with the sequence alignment could be explained by OGG1 being a newly discovered homolog protein of MUTYH. Because not many sequences are available for OGG1 on UniProt and NCBI yet, sequence alignments may not be complete for MI calculations.

The APEX1 versus MUTYH experiment showed a positive linear relationship between z-score and number of species, much similar to what was observed in the experiment comparing two domains of MUTYH and MutY (Figure 10). But differences in the level of confidence between interactions of APEX1 and MUTYH and two domains of either MUTYH or MutY exist. This difference is explained by the two domains of MutY and MUTYH being guaranteed to have the same species, coming from the same protein. However, APEX1 is not part of the MUTYH protein therefore the confidence interval of functional interactions between the two proteins can start at a lower point. The main takeaway with this experiment was that positive relationships between the functional interactions of the two proteins, MUTYH and APEX1, exist. Much different from results observed between functional interactions of OGG1 and MUTYH which were inconsistent and had very low overall z-score.

It is of note that no functional interactions between APEX1 and MUTYH were observed at the 50 species. Meaning that in order to correctly make assumptions about whether interactions exist between two proteins, it is essential to test them at different numbers of species. However, it is difficult to find more examples of MUTYH and its partner proteins APEX1 and OGG1 which limited the analysis of functional interaction dependency on the number of species. In contrast to mammalian DNA repair proteins such as MUTYH and its partner proteins, databases contain vast numbers of bacterial and archaea sequences. Therefore, I hoped that the MI analysis would lead to more confident results for MutY and its partner proteins, XthA and Nfo.

Initially I hypothesized that interactions between MutY and its partner proteins would be similar to interactions of MUTYH and its partner proteins. To check the hypothesis, functional interaction between MutY and its two partner proteins, XthA and Nfo were checked across 50- 250 species. MutY and XthA showed to have no functional interactions that could be detected by MI. Lack of functional interactions between the two proteins is accurate because the results were consistent across different numbers of species, Figure 11. Next, interactions between MutY and Nfo, another one of its partner proteins, were tested. Functional interactions between the two proteins were slightly seen at 100 level species but were more clearly observed at 250 level species as shown in Figure 12. The existence or lack of functional interactions between MutY and its partner proteins, XthA and Nfo rejected the hypothesis that MutY and its partner proteins interact the same way as MUTYH interacts with its partner proteins, although both are homologous proteins of each other. However, having more bacterial sequences available in the databases can give us more assurance of the resulting MI distributions.

## Conservation of Functionally Important Regions Across Species through Evolution

The Mutual Information Calculator relies on variation in amino acid identity to detect covariation. Absolutely conserved positions in the sequence alignment are guaranteed an MI score of zero as are positions with a high degree of variation that does not correlate to variation in a second position in the other protein. The "winning" MI scores that are significantly higher than the MI cutoff are interesting because these suggest functionally important interactions that are partially, not absolutely conserved across evolution.More the DNA repair proteins I studied, we have outside information about functionally important regions and can view these in the experimentally determined protein structure. To observe whether functionally important regions in a protein structure correspond with functional

interactions detected by the Mutual Information calculator, I examined winning positions in the structures of MUTYH and MutY.

Four amino acids are known to interact with iron-sulfur clusters in both MUTYH and MutY proteins. Cys261, Cys 268, Cys 271, and Cys 277 interact with an iron-sulfur cluster in MUTYH and Cys198, Cys 205, Cys208, and Cys 214 interact with the iron-sulfur cluster in MutY. These critical Cys amino acids were cross-referenced with the winning positions identified by high MI scores when analyzing the NTD and CTD for MUTYH and MutY. I found that all cysteine residues were amazingly well conserved and retained across 500 and 5000 species, explaining why the Cys positions were not flagged by high MI scores. Interestingly, the amino acids located right before and after the cystines occupied winning positions as indicated by high MI scores consistent with the functional importance of the iron-sulfur cluster illustrated in Figure 13. It is of note that interactions between the iron-sulfur structure and the CTD were observed in the MI distributions obtained with a larger number of species; with a smaller sample of species, 50 for MutY and 100 for MUTYH, these interesting interactions were not detectable. Furthermore, amino acids around the cysteines which were recognized from experiments with 500 and 5000 species, which are located in the NTD of both MutY and MUTYH showed interactions with the CTD which is very far away in the structures of these proteins.

A zinc ion located in the interdomain connecter region (IDC) of MUTYH, is functionally important for the proper engagement and position of the two functional domains (Nuñez et al. 2018). In order to see whether the previous observations from SF4 structure stand true for all amino acids interacting with functionally important groups in a given protein, I compared the MI "winner" data that was obtained from the MI calculator to His 56 and Cys 300, which are the two amino acids that directly interact with the zinc ion in the reference structure of MUTYH (PDB ID 7EF9). As expected, His 56 retained absolute conservation across species, explaining why it is not found in the winner positions flagged by the MI calculator. However, a modest degree of variation for Cys 300 across 500 and 5000 species allowed for inclusion of this cysteine in the winner positions. As seen in Figure 14, Cys 300 in the reference sequence CALNT in the 7EF9 structure, was analyzed to not only have a direct interaction with the zinc ion, but it also was involved in presumably functional interactions with several residues in the CTD as suggested by high MI scores calculated across 500 and 5000 species in MUTYH. Personally, it was really cool to see how MI scores correlated with functionally important regions of protein structure!



Figure 9. MI Distribution graphs between the MUTYH and OGG1. Graphs A to C: Looking at mutual interactions across 50 to 250 species. Graph D: Linear relationship between Number of Species and Z-score. As the number of Species increases, confidence in MI also increases



Figure 10. MI Distribution graphs between the MUTYH and APEX1. Graphs A to C: Looking at mutual interactions across 50 to 250 species. Graph D: Linear relationship between Number of Species and Z-score. As the number of Species increases, confidence in MI also increases

#### M info MUTYH vs APEX1 100



Figure 11. MI Distribution graphs between the MutY and XthA. Graphs A to C: Looking at mutual interactions across 50 to 250 species.



Figure 12. MI Distribution graphs between the MutY and Nfo. Graphs A to C: Looking at mutual interactions across 50 to 250 species. A: Random information (RAND) bars match with that

of mutual information bars (INFO) showing that no functional interactions exist between the two proteins. B,C: Functional interactions are becoming visible as more species are used. Graph D: Linear relationship between Number of Species and Z-score. As the number of Species increases, confidence in MI also increases

MUTYH and its GO Pathway Partner Proteins

Next, I wanted to see whether 3D structures of MUTYH and itss partner proteins showed functionally important elements that were linked by MI scores. At both 100 and 250 species, two amino acids in APEX1 showed functional interactions with CTD of MUTYH protein Figure 15. Other amino acids were also recognized by MI to be interacting with MUTYH, however they could not be visualized in ChimeraX because they did not code for secondary structures. Interestingly, OGG1 behaved similarly to APEX1 in that the residues linked by MI had functional interactions with the CTD region of MUTYH. Figure 16. C- terminal Domain of MUTYH is essential in the function of folding over the DNA to recognize lesions (Russelburg et al. 2019). These structural interactions can show that MUTYH partner proteins specifically interact with the region of MUTYH that is essential in the GO DNA Repair Pathway.



Figure 13. Residue interactions around Fe-S Cluster in MUTYH. Left: Close up view of the residues surrounding the Fe-S cluster, Cysteines are color coded in red, while residues indicated by winning MI scores are highlighted in green. Right: Zoomed out view. For simplicity, showing interactions between Leu 267 and residues in the C terminal domain both color coded in red. CTD in green and NTD in medium purple. Figure prepared with coordinates from PDB 7EF9.



Figure 14. Cys 300 of MUTYH interacts directly with Zinc ion and the CTD. Cys 300 was flagged as a high scoring "winner" in the MI distribution when paired with several residues found in the CTD. The crystal structure shows a direct connection to the zinc ion. Structure links MI scores to functionally important elements like metal ion binding sites.


Figure 15. Interactions between APEX1 and MUTYH. Pro59 and Asn 259 in the 6BOR structure of APEX1, the red box, interact with the CTD region of MUTYH 7EF9 structure in the blue box. Functional amino acids colored in red. CTD of MUTYH in light green.



Figure 16. Interactions between MUTYH and OGG1. residues in the 2XHI structure of OGG1 that code for secondary structures and have high MI scores (shown in red box) interact

with the CTD region of the 7EF9 structure of MUTYH (shown in blue box).

#### Discussion

The role of Deoxyribonucleic Acid (DNA) has been discovered and researched for many years now. In 1944, Oswald Avery, Colin MacLeod, and MacLyn McCarty showed the uptake of a pure DNA in mice from a virulent bacterial strain (Avery, Macleod, and McCarty 1944). This indicated that this strain could confer virulence to a nonvirulent strain, meaning DNA influences cell properties and their outcomes. Later, in 1952 Alfred Hershev and MarthaChase proved that genes are made of DNA by radiolabeling bacteriophage protein coats and DNA core separately, finding that it is DNA that is used in a bacteriophage infection (Hershey and Chase 1952). This added another piece to the puzzle, demonstrating that DNA is the source of hereditary information. In 1953 Rosalind Franklin would experimentally produce X-Ray crystallography images of what appeared to be a double-helical structure of DNA. Following these findings, it was James Watson and Francis Crick who, using Franklin's images, would develop what has become the modern Watson-Crick double-helical model of DNA (Watson and Crick 1953). These scientists and their experiments were the beginning of the understanding of our own genome and cell biology as they would explain DNA as genetic material and create its secondary structure.

It was not until over 25 years after these discoveries that advanced technologies and sequencing methods enabled the study of the DNA structure and its function at a molecular level (Gauthier et al. 2019). The National Center for Biotechnical Information (NCBI) famous search tool, the Basic Local Alignment Search Tool (BLAST) was only discovered in the 1990s and the first genome shotgun sequences were not included in the GenBank tool until 2002 (K. Smith 2013).

It has only been approximately 20 years since access to a large database with many sequences has become available to researchers all over the world. As the world is progressing towards more technological advances in the field of research and medicine, the access to a large number of datasets has allowed researchers to make many new assumptions about the behaviors of proteins, enzymes, and nucleotides, and has given them the tools necessary to analyze, visualize, and answer This explosion in the these questions. advances of bioinformatics research has and will continue to have an evolutionary impact on the medical and pharmaceutical world targeting specific amino acids in a sequence and observing distinctive cellular behaviors at the molecular level have become increasingly essential for the improvement of our own understanding of cell biology and science as well as the development of modern medicines to battle fatal diseases such as cancer.

#### MI Score as an Indicator of Coevolution

Mutual Information (MI) Calculator computes mutual codependency between two variables, two proteins for example, meaning as the measure of the strength of association between two proteins increases, codependency between the two proteins increases also. The MI Score is the strength of association between these given proteins and since the experiments focus on the behavior of protein domains and partner proteins, the MI Score can be interpreted as coevolution and codependency between the two proteins (Little and Chen 2009; Kinney and Atwal 2014).

Using bioinformatics and analyzing the sequences from thousands of species, many interesting conclusions have been made from MutY, one of the DNA repair proteins. Bacterial MutY and its eukaryotic ortholog, MUTYH specifically recognize OG:A mispairs, where as a result of mutation an oxygenated-guanine is in place of adenine, in DNA as shown in the results section.

To emphasize some key points of my research it is understood that as the number of species analyzed increases, the difference between Random and Information MI scores also increases to increase the confidence interval of the results. Meaning as the difference between the two scores increases, covariation and codependency between the two values also increases. Secondly, it was concluded that MutY and MUTYH do not behave identically with their partner proteins despite being homologous proteins. And finally, it is important to note that while functional interactions were observed in the form of MI scores, the visualization of the proteins in the modeling software, ChimeraX revealed interesting behaviors between the amino acids that have evolved together through time.

#### **Coevolution for DNA Repair Partner Proteins**

MutY's and its eukaryotic ortholog structure MUTYH have the ability to recognize and prevent mutations caused by the 8OGuanine:adenine (8OG:Aa) mispair on the double-stranded DNA structure. As OGG1 recognized and repairs OG:C lesion, MUTYH recognized and repairs OG:A lesions in the GO DNA Repair Pathway. This complex pathway is then continued by APEX1 which excises the generated AP site opposite of the 80G strand to initiate repair (Tominaga et al. 2004; Russelburg et al. 2019).

The GO DNA repair pathway is complex and it is essential for MUTYH to work closely with its partner proteins to allow this repair mechanism to work smoothly. This tight interaction between MUTYH and OGG1 as well as MUTYH and APEX1 was visualized by modeling the interesting amino acids as indicated by MI score calculations in ChimeraX. As mentioned in the results section, most residues indicated by the MI score in both APEX 1 and OGG1 experiments, did not code for secondary structures. However, the few that were visible showed selective interaction with the C-terminal domain (CTD) of MUTYH. As it was published in a research article, the presence of the MUTYH CTD prevents OGG1 and APEX1 from inappropriately processing its substrate and product. If MUTYH did not have the ability to prevent OGG1 from acting on its product, then improper excision of 80G opposite of the AP site could cause loss of information bases located on both strands of DNA (Tominaga et al. 2004). Meaning that through many years of evolution, certain amino acids in APEX1 and OGG1 have coevolved mostly with the CTD of MUTYH through billions of years to perfect this collaborative mechanism of DNA base repair that can help save millions of people from lung, rectal, and colon cancers if studied more deeply.

Furthermore, after running three experiments involving 100, 500, and 5000 species of MUTYH sequences to study MI Score and codependency between the two domains of the protein, Nterminal domain (NTD) and CTD, it was revealed that a specific residue in the CTD loop sits in an anti-conformation to the NTD and is positioned in an area with the most interactions with the NTD. This interaction was not previously expected as the residue sits farther away from the linker region between the CTD and NTD region, meaning that intramolecular interactions were possibly not the results of this interaction. Visualization of this residue, however, led to the discovery of the intramolecular interaction between the two domains. In other words, although these regions are far from each other, they are possibly interacting intramolecularly on separate MUTYH proteins as displayed in Figure 17.

Understanding the coevolution of proteins for medical application

This knowledge was observed using a newly advanced bioinformatics approach and 3D visualization enabled by ChimeraX and has opened many doors into not only understanding why certain regions of partner proteins or domains might interact with each other, but also allow us to target specific amino acids in a given protein and conduct more focused research to understand the reasoning behind 80G:A base repair mechanism and create more promising assumptions for pharmaceutical conduct in medicine. For instance, in 2017, Banda and colleagues showed that the lack of MUTYH and OGG1 enzymes cause spontaneous tumor formation when compared to mice that retained the OGG1 enzyme. Furthermore, it was revealed that increased oxidative stress and 80G formation had become hallmark characteristics of neurodegenerative Alzheimer's, disorders such as Huntington's, and Parkinson's diseases (Banda et al. 2017). However, specific mechanisms of how and why MutY and MUTYH might be related to such different types of diseases are still unknown. Using bioinformatics as a tool of modern research, it is now possible to observe and explain the interactions and behaviors of repair proteins and unlock many doors to designing molecular mechanisms and forming

significant predictions. Bioinformatics will also play a key role in targeting more effective substrates in modern medicine using an evolutionary perspective.

#### MutY and its GO Pathway Partner Proteins

Interestingly, out of all the experiments conducted to check functional interactions between MutY and its partner proteins, only the Nfo protein at the 250 species level accurately returned a positive indicator of coevolution between the two proteins via the mutual information score results (Table 1). Functional interactions between Nfo and MutY could not be clearly distinguished at the 100 level species as mutual information score was not consistent between two separate experiments which were run for this relationship. Furthermore, neither of the 250 species or 100 species level experiments between the two proteins could be visualized in ChimeraX as reference structures could not be recognized by MI calculator to aid with visualization. However, MI calculator was inspected and it was concluded that although debugging the final steps of MI calculator might become necessary to avoid such issues in the future, they do not disturb the accuracy and data when producing the MI distribution graphs. Therefore, using the graphs it is clear that coevolution does exist between the two proteins, Nfo and MutY, at the 100 and 250 level species, however they cannot be visualized at this time.

#### Prospectus

Looking forward, further improvements to the MI calculator performance in identifying reference structures must be done to increase the chances of visualizing the interesting residue behaviors in a 3D environment such as ChimeraX. Future research analyzing the Functional properties of amino acids as indicated by a MI score will be necessary to draw more precise conclusions about other molecular interactions as was observed in these experiments. More experiments must be conducted to check these functional interactions between MUTYH and MutY and their respective partner proteins. This research should include a large scale of species, more than I was able to analyze due to time constraints.



Figure 17. Intermolecular interactions between NTD and CTD of MUTYH on PDB 7EF9 structure. CTD shown in light green and NTD shown in medium purple. I 351 located in the loop of CTD seems to be the residue interacting the most with the NTD region. Interacting residues on both NTD and CTD colored in red for visibility. Few residues on NTD labeled for reference. Dashed lines indicate interaction between the residues.

# Methods

Obtaining Sequences from Databases

FASTA sequence files for experiments that required 250 or less species of MUTYH, MutY, APEX1, OGG1, XthA, and Nfo, were obtained from UniProt. Initially the older version of Uniprot offered little filtering options and was therefore less user friendly but later updates allowed me to obtain

better results. In order to obtain sequences for MUTYH and MutY proteins from UniProt I searched for the name of the protein in the search bar, selected for all of the sequences using the "select all" option on the top left side of the results pages, and visually identified and unselected sequences that seemed to not have been derived from the desired protein or if the sequence lengths seemed to be outside of the normal range. The sequences were then added to the "basket", a data holder tool on UniProt. The "basket" tool allowed me to download the sequences in the desired format, which was FASTA so that I could further edit the sequences as needed. The older version of UniProt did not allow me to select more than 200 sequences at once which was one of its main limitations.

A newer version of UniProt became available about midway through my work, and allowed for better sequence retrievals for the second round of experiments looking at partner protein interactions. Using the Advanced Search of the UniProtKB Database on the UniProt official website, UniProt.org, the desired protein's name was inserted, MUTYH, MutY, APEX1, OGG1, XthA, or Nfo, in the field titled "Gene Name [GN]". Name of the protein that I explicitly did not want to be mixed in was inserted in the field titled "Gene Name [GN]" categorized as [NOT], which was only explicitly reserved to eliminate MutY sequences from our MUTYH search, and the target sequence length was inserted in the "sequence length" field categorized as [AND]. Sequence length of 400-600 amino acids was applied for MUTYH, 300-500 amino acids for the MutY experiments, 300-450 amino acids length was applied for OGG1, 300-400 amino acids length was applied for APEX1, and 200-300 amino acids length was applied for XthA and Nfo proteins. Figure 18 shows an example of the search bar with options and filters applied to find MUTYH sequences.

FASTA sequences for thousands of species. It was obtained from NCBI's BLASTP tool which I had reserved for retrieval of large datasets. The browser for the search tool was quickly accessed using the following url: https://blast.ncbi.nlm.nih.gov/ Blast.cgi?PROGRAM=BLASTP&PAGE\_TYPE=Blast Search&LI NK\_LOC=blasthome

The link was obtained by combining the base url, https://blast.ncbi.nlm.nih.gov , with Blast.cgi, which is the general blast tool, and using BLASTP, as the main program for looking at proteins. The url was closed by referring to the home page of the search tool using the page type description PAGE\_TYPE=BlastSearch&LINK\_LOC=blasthome (Sayers 2021; Kans 2022; "EDirect\_EUtils\_API\_Cookbook" [2016] 2022).

In order to get a large number of sequences for MutY and MUTYH, accession IDs of the proteins needed to be inserted inside the BLASTP search box. Accession IDs were obtained by initially searching the desired protein from the desired organism, e.g MUTYH of Mus musculus (mouse) in the protein database of NCBI, and clicking on the result that is representative of the desired protein from the desired organism. After inputting the accession ID into the search tool, the algorithm parameters were adjusted such that max target sequence was set to 500 or to 5000, except threshold was kept at 0.05, and low complexity regions were filtered out. To gain access to sequences with more taxonomic depth, Clustered nr was selected as the database for BLASTP. As explained in the results section, only accession IDs of MUTYH, MutY and OGG1 were needed for the experiments which are shown in Table 2. Examples of how the search was performed can be found in Figure 19.

#### Alignment

PROMALS3D, an online alignment tool, was used to align the various sequences. PROMALS3D uses an advanced technique to create Multiple Sequence Alignments (MSA) by combining information gained from BLAST and PSI-BLAST searches to identify homologous sequences, predicted secondary structures, and retrieve 3D structures to increase the quality of the alignments (Pei and Grishin 2014). PROMALS3D outputs different types of alignments, and includes the option to view the alignments as a single line or in brackets of certain lengths. Examples of the color-coded alignment and the alignment in the form of a FASTA file is shown in Figure 20. I visualized these alignments using the input format of the FASTA file option which allowed me to copy and paste the alignments into a text editing software, Sublime Text. From here I was able to further edit these sequences to an appropriate and efficient form before running them in the MI calculator.

## **Preparing and Cleaning Data**

Separate approaches needed to be taken to clean the FASTA sequences that were retrieved either using UniProt or NCBI as the database. In both cases, the ultimate goal is to obtain two necessary files with correct formatting that can be easily read in the preprogrammed MI calculator. The first file must contain a list of taxonomic identifiers, or taxon IDs of all the species in the experiment. FASTA headers extracted from the UniProt include protein names, scientific names of the organisms, names, taxon IDs. and database gene identifications. Taxon IDs are introduced with the acronym, OX=, in the header. Therefore this information can be used to create a list of all the OX values and use it as the species list. The next file must contain the aligned sequences without the headers as will be further explained below.

### **UniProt: Preparation of the Species List**

Tsh Command Line Tool and the "grep" function allowed me to create a list of taxon IDs which will often be referred to as the list of species throughout the thesis. The "grep" command is a Command Line tool which searches for regular or repetitive expressions in the headers of the FASTA files and sends them into a separate temporary file. This temporary file was then visualized and coded to remove all matched white spaces or gaps, using the

code" :g/\s\+/s//\r/g " where "g" is a global search flag marker that uses RegExp search to look for a pattern throughout a single string, "\s" matches all white spaces, "\s+" counts for one or more spaces, and "r/g" replaces all occurrences. This code was then closed using the write and quit ":wq" function. This sequence of codes creates a list of all the header information, each presented at a single new line. Next, all the taxon IDs were extracted by using the grep function and looking for the character pattern "OX=". Since the pattern "OX=" occupies three character positions, the grep "OX=" command was piped, and the "cut" command further extracted and maintained all characters at positions 4 to 20 by "cut -c 4-20 file.name". This only retained all the following integer values specified by "OX=" in the list while also omitting the "OX=" pattern. The end result is a simple file that shows the list of gathered taxon IDs and no other data.

| Advanced Search                          |                                    |        |        |  |  |  |
|--|------------------------------------|--------|--------|--|--|--|
| Searching in                             |                                    |        |        |  |  |  |
| UniProtKB                                |                                    |        | *      |  |  |  |
|  | Gene Name [GN]                     |        |        |  |  |  |
| ▼ Gene Name [GN] ▼                       | MUTYH                              |        | Remove |  |  |  |
|  | Gene Name [GN]                     |        |        |  |  |  |
| NOT ▼ Gene Name [GN] ▼                   | mutY                               |        | Remove |  |  |  |
|  | From                               | То     |        |  |  |  |
| AND <ul> <li>Sequence length </li> </ul> | 400                                | 600    | Remove |  |  |  |
| Add Field                                |                                    | Cancel | Search |  |  |  |
| Type * in the search box to search for a | all values for the selected field. |        |        |  |  |  |

## Figure 18. UniProt Advanced Search tool

The alignment that is produced from PROMALS3D includes the structure sequence, which increases the total number of the species by one. Since the MI calculator uses a pattern matching algorithm to match the number of species to the number of input sequences, the species list needed to be further adjusted by adding the alignments reference structure taxon ID to the list, matching the total number of species to the total number of sequences. This list was then titled "species" in order for it to be recognized by the MI script in R Studio.

# UniProt: Preparation of the NTD and CTD Sequences

Two files needed to be created by cutting the aligned sequences obtained from PROMALS3D into two separate files for the N-Terminal Domain

(NTD) and the C-Terminal Domain (CTD), respectively. To locate the sequence regions from which the two domains needed to be cut, it was necessary to visualize the reference structures of the MUTYH and MutY proteins in ChimeraX, an extensible program for interactive visualization and analysis of molecular structures (Pettersen et al. 2021). From here I located the amino acids at the two ends of the linker region which separates the two domains which surround the DNA doublehelix. The resulting residue sequences were recorded to be the beginning and ending of the NTD and CTD domains. For MUTYH, the NTD was determined to be from glutamine with the sequence:QASVS, to phenylalanine with:GVANF. The CTD was determined to be from arginine of REEYS to glutamine of YEDHRQ in MUTYH mouse reference structure, 7EF9. For MutY, NTD was determined to be from proline of PAREF to leucine of VAEEL and CTD was determined to be from lysine of KQVPL to alanine of YKEWA in the reference structure, 6U7T. from Geobacillus stearothermophilus. After downloading a new alignment that was obtained from PROMALS3D into a text editor, the position of the beginning and ending sequences of the two domains were obtained in the reference structure's alignment which is often the last sequence in the alignment. Each alignment contains a different number of gaps therefore the process of obtaining the position of

the beginning and end of the domains had to be repeated after every alignment. After obtaining the position values, "cut" command in tsh Command Line Tool removed the headers from the alignments and cut the two domains based on the positioning in the reference structure.

| Protein | PDB   | Chain | Resolution | Method | Organism                              | Taxon ID | Accession<br>ID    |
|---------|-------|-------|------------|--------|---------------------------------------|----------|--------------------|
| MUTYH   | 7EFF9 | A     | 1.97 A°    | Xray   | Mus<br>Musculus                       | 10090    | NP_00104<br>1639.1 |
| APEX1   | 6BOR  | A/B   | 1.84 A*    | Xray   | Homo<br>Sapiens                       | 9606     | Not used           |
| OGG1    | 2XHI  | A     | 1.55 A°    | Xray   | Homo<br>Sapiens                       | 9606     | NP_00134<br>1578.1 |
| mutY    | 6U7T  | A     | 2.00 A*    | Xray   | GeoBacillus<br>Stearothermo<br>philus | 1422     | WP_01537<br>3971.1 |
| xthA    | 1AKO  | A     | 1.70 A*    | Xray   | Escherichia<br>Coli                   | 83333    | Not used           |
| nfo     | 4K1G  | A/B   | 1.90 A*    | Xray   | Escherichia<br>Coli                   | 83333    | Not used           |

Table 2. Reference protein structures

| NIH) National Library of Medicine  | - Algorithm parameters   |  |  |
|--|--|--|--|
|  | General Parameters   |  |  |
| BLAST ® » blastp suite Home  | Max target<br>sequences + 5000 - Sect the maximum number of aligned sequences to display @                           |  |  |
| blastn blastp blastx tblastn tblastx   | Short queries Z Automatically adjust parameters for short input sequences ()   |  |  |
| BLAGTP programs search protein databases using a protein query more  | Expect threshold 0.05  |  |  |
| Enter accession number(s), gi(s), or FASTA sequence(s) 😧 Claw Query subrange 😧   | Word size 6 v 0  |  |  |
| NP_00/941038.1   | Max matches in a 0 0   |  |  |
| то   | Scoring Parameters   |  |  |
| Or, uplead file Choose File No file chosen   | Matrix BLOSUMB2 Y  |  |  |
| Job Title  | Gap Costs Existence: 11 Extension: 1 ¥ 🚱   |  |  |
| Align two or more sequences 🛛  | Compositional Conditional compositional score matrix adjustment V  |  |  |
| Choose Search Set  |  |  |  |
| Databases  Standard databases (m etc.):  Standard databases (m etc | Filter Low complexity regions 🖗  |  |  |
| Compare Select to compare standard and experimental database 0   | Mask  Mask for lookup table only  Mask lower case letters  |  |  |
| Experimental   |  |  |  |
| Database  + Clustered rn(rr_clustered)  • 0  | BLAST Stearch database nr_clustered(experimental) using Blastp (protein-protein BLAST)  thew results is a new vietow |  |  |

Figure 19. NCBI BLASTP search tool. Left: inputting the desired protein accession ID as indicated in Table 2 into the search tool and choosing Experimental Database as the search database. Right: Adjusting the Algorithm Parameters to

increase the value of Max target sequences and adding a filter to remove regions with Low Complexity.

| tr Q8VN70 Q8VN70 HELPX Ad  | 1   | INAPYEVYISEVMSQQTQISTVVERF                            | 53           |
|--|---|---|--------------|
| tr Q8VN75 Q8VN75 HELPX Ad  | 1   | INAPYEVYISEVMSQQTQISTVVERF                            | 53           |
| tr Q8VN77 Q8VN77 HELPX Ad  | 1   | INAPYEVYISEVMSQQTQINTVVERF                            | 53           |
| tr Q8VN79 Q8VN79 HELPX Ad  | 1   | INAPYEVYISEVMSQQTQINTVVERF                            | 53           |
| tr A0A0S2LTY5 A0A0S2LTY5   | 1   | INAPYEVISEVMSOCTOISTVVERF                             | 53           |
| tr A0A0S2LU50 A0A0S2LU50   | 1   | INAPYEVISEVMSOOTOINTVVERF                             | 53           |
| tr A0A0S2LU52 A0A0S2LU52   | 1   | INAPYEVISEVMSQQTQINTVVERF                             | 53           |
| tr A0A0S2LU60 A0A0S2LU60   | 1   | INAPYEVYISEVMSOOTOINTVVERF                            | 53           |
| tr A0A0S2LU61 A0A0S2LU61   | 1   | INAPYEVYISEVMSQQTQINTVVERF                            | 53           |
| tr A0A0S2LU63 A0A0S2LU63   | 1   | INAPYEVISEVMSOOTOINTVVERF                             | 53           |
| tr A0A0S2LU64 A0A0S2LU64   | 1   | METLHNALLEWYEECGRKDLPFRNLKGINAPYEVYISEVMSQQTQINTVVERF | 53           |
| tr A0A0S2LU76 A0A0S2LU76   | 1   | INAPYEVYISEVMSQCTQINTVVERF                            | 53           |
| >ap_P\$3447_MUTY_GEOSE_Adea<br>MTR-<br>SKYNELLMAVKEVKITKYGGKY7000PDEFSRLKOVQPTYVGAV<br>XTENROAMTMALTELGALVCTYBAPGCLCPVQAVCQAFAG<br>>p_A08567_UUTY_TYCG2_Adea<br>RRAKELBECAVYLASETDOVYPROVOTLJTLF0GAYTAMAV<br>RRAKELBECAVYLASETDOVYPROVOTLJTLF0GAYTAMAV | LSLAYGVPEPA<br>VAEE                                       | ПТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИ               | 'NG<br>SAGP- |
| rep_031584_MUTY_BACBU_Ades<br>HOVLEDK<br>REWRIELGANVERVRQETGOIV970EX0F0GLKOVQFTFKGAV<br>SEKKRETNGGLHELGALLCTVFVGFCALFYQGURGAFTKG<br>   | LSIAYNKPIPA<br>TERE<br>SHQKIWKMAGE<br>LSLSLGKHFPI<br>SWAL | - LOP OTHER LIPTEND                                   | :QV          |

Figure 20. PROMALS3D alignment of MutY sequences across 100 species. Top: Color coded alignment – Representative sequence is color coded as magenta with all other sequences following the magenta being aligned according to the positioning of the representative sequence. Red color code represents alpha-helix secondary structures as shown and blue represents beta- strand, not shown. Bottom – Alignment visualized as the input FASTA format.

# NCBI: Preparing the Species List, NTD, and CTD Files

During the conduction of the experiments, I had to find a quicker way to clean and prepare data lists for the MI calculator, therefore I wrote two sets of cleaning codes with the help of Dr. Martin Horvath that could clean and filter input files and alignments obtained using both UniProt and NCBI as databases. The codes were written using R studio and a summary and description of the codes can be seen in Appendices.

In order for the script to function properly, it

is essential to download the correct files from the BLASTP results page. To create the species list, the "Cluster Table (csv)" file needs to be downloaded and the "Clusters (FASTA)" file needs to be obtained for input into PROMALS3D, as previously explained.

The script for creating the species list as well as the files prepared using the alignments use a mixture of commands provided by the packages: ggplot2, dplyr, readr, stringr, tidyverse, tidyr, and tibble. The base function of R Studio and its "readr" packages allowed the creation of the list of taxon ids from the "Clusters Table (csv)" file that was obtained from the BLASTP tool. The script is coded to first read the file into R using read.csv, and following by capturing the specific rows that obtain the taxon IDs conveniently located in the column titled "Taxid " and sending them into a new data frame. The data frame is then renamed to "species", to be recognized and read in the MI Calculator script. Additionally, the organism ID of the reference structure is added at the end of the species list using a base function of R Studio to match the total number of species to the total number of aligned sequences (See Appendix 1 and Appendix 2).

To run large numbers of aligned species in the MI calculator in a timely manner, it is necessary to reduce the number of gaps present between each sequence, as the MI calculation relies on the string-wise comparison of the two files provided. Part two of the script found in Appendix 3 shows the loop which was written to calculate the number of gaps in each substring position, as counted in a columnar fashion. This only retained substrings where only 20% of gaps were present. This was done by using the str\_sub command available in the "stringr" package, which specifically analyzed and counted the occurrence of gaps as shown with the character "-" in the data displayed as a vector of characters.

Furthermore, str\_locate and str\_sub ,from the "stringr" package in R Studio, located and cut the beginning and ending positions of the NTD and CTD. The beginning and ending sequences of the two domains were found as previously explained in the UniProt Data preparation section (see Appendix 4).

#### **Running the MI Calculator**

Mutual information (MI) calculator that allowed these experiments was previously coded by Dr. Martin Horvath to calculate the rate of interaction by detecting patterns of change that co- exist in the two given variables, two proteins or two domains of protein. Given this algorithm to compare and match, MI returns an MI score for mutual interaction with patterns. Higher MI Score indicates that statistically significant interactions between the two variables exist.

The MI score is calculated by conducting two separate experiments for each data set, one of paired species which returns mutual interactions MI score, and one of unpaired or scrambled species. This results in the control experiment's MI score. The graphs produced from these two experiments will be combined into a single histogram, the MI distribution graph, and the difference thereof will be the statistically significant and interesting mutual interactions between the two proteins that are not by random chance (Appendix 5). The point at which the scrambled species MI score stops on the MI distribution graph is referred to as the MI cutoff. The MI cutoff has been used to observe where mutual interactions start and how the confidence interval, calculated as a Z-score, changes across different numbers of species. The plots have been made using the "ggplot2" package available in R, and calculations were conducted using a mixture of "tibble", "readr", "tidyr" and "tidyverse" packages. The R program encoding the MI Calculator is available request upon to martin.horvath@utah.edu.

#### Acknowledgements

I would like to acknowledge and thank my supervisor Dr. Martin P. Horvath for his continuous support and guidance throughout my Honors Thesis journey and for allowing this work to become possible. I would also like to thank Dr. Horvath for his patient encouragement as I learned the code writing process.

I am very appreciative of all the supervisors and professors who have guided me through the ups and downs of completing the Honors degree, especially Dr. Naina Phadnis and Dr. Virginia Solomon for their continuous patience and advice throughout my journey, and to Dr. Thomas Richmond and Dr. Holly Sebahar for opening endless doors of opportunities to help me continue with research despite difficulties.

I am also grateful to all Horvath Lab members for helping me understand the different aspects of MutY and MUTYH DNA repair mechanisms based on their respective knowledge and expertise. And I would especially like to thank Nicky Loyola for his awesome help with proofreading the thesis, and Harin Srinivasan for referring me to this amazing lab.

And last but not least, I would like to thank my family, my parents and my sister, who made great sacrifices to allow me to continue education and research in the University that I had only dreamt about for years. I would like to thank my family for their endless support and encouragement through my undergraduate years, for helping me take long steps towards my goal, and for staying awake with me the nights that I had to stay awake to study.

This work was supported by the National Science Foundation Award 1905249.

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#### APPENDIX

1: UniProt: Creating the species list and removing gaps

```
#Read the input file that was downloaded from UniPro
x <- read.delim("MutY 251 input.fasta", header = FAI</pre>
stringsAsFactors = FALSE)
#Turning the data frame into a vector value
x.new <- x$V1
#To grab just the headers, indicated by character ">
x.new <- x.new[grepl(pattern = ">", x = x.new , fixe
#locate and extract all words (or continuous string
characters) that contain OX
B <- data.frame(str extract all(x.new, "(?<=\\b)OX['</pre>
#Switch Rows and Columns and rename column
OX <- data.frame(data.frame(t(B)))</pre>
colnames(OX) <- "X1"
#Turning data frame into vector
Taxid <- OX$X1
#grab only the numbers from the OX value list, rename
and write out.
Species <- data.frame(parse number(Taxid))</pre>
colnames(Species) <- "species"</pre>
Species[(nrow(Species)+1), "species"] <- 1422 # Whe:</pre>
homo sapien taxon id. Change as needed
#Write out the species list.
write.table(Species, file = "MutY 251 OX.species", :
FALSE, col.names = TRUE, quote = FALSE)
#Read the alignment file obtained from Promals3D int
remove headers.
promals3D alignment <-
read.delim("protein promals3D alignment.fasta", head
stringsAsFactors = FALSE)
Alignment <- data.frame("alignment" = character())</pre>
indexT <- as.integer(2)</pre>
```

```
while (indexT <= nrow(promals3D_alignment)) {
Alignment[indexT/2, "alignment"] <- promals3D_alignm
"V1"] # such that headers are located on every other
remove headers every other line needs to be removed</pre>
```

```
indexT <- 2 + indexT
```

```
} #Removing header and building aligned sequences.
#Write out the new file of alignment without the heat
colnames(Alignment) <- "proteinA" # adding Column heat
readable in R
write.table(Alignment, file = "MutY_251.promals3D",
FALSE, col.names = true, quote = FALSE)
```

#### 2: NCBI: Creating the Species List

```
#Extract rows 1-250 (for 250 species) where species
information is located in a column with heading tax:
shown for MUTYH protein.
#Replace the column heading from "taxid" to "specie:
x <- read.csv("MUTYH 250 info.csv")</pre>
species.A <- data.frame(x[1:250, "Taxid"])</pre>
colnames(species.A)
colnames(species.A) <- "species"</pre>
#check that the column heading has been changed succ
colnames(species.A)
#Add the reference structure's taxon ID at the end of
species list.
species.A[(nrow(species.A)+1), "species"] <- 10090 +</pre>
is mus musculus taxon id
#Write the file out.
write.table(species.A, file = "proteinA OX.species",
FALSE, col.names = TRUE, quote = FALSE)
```

#### 3: Removing Gap Rich Columns from the Alignment Files

```
## Recognize gaps in the data, remove columns where
80% is gap
indexA <- as.integer(1)</pre>
```

```
NewDashCount <- data.frame("DashCount"= numeric(), '</pre>
logical(), "position" = as.integer())
while (indexA <= str_length(Alignment[1,"alignment")</pre>
 temp <- str sub(Alignment[, "alignment"], indexA, :</pre>
 NewDashCount[indexA, "DashCount"] <- sum(str count</pre>
 if(NewDashCount[indexA, "DashCount"] < 50){ #where</pre>
250. Change as needed
  NewDashCount[indexA, "keep"] <- TRUE</pre>
 } # counting dashes and removing brackets more than
 if(NewDashCount[indexA, "DashCount"] >= 50) { #where
of 250. Change as needed
  NewDashCount[indexA, "keep"] <- FALSE</pre>
 } # counting dashes and removing brackets more than
(or retaining regions with only 20% gap)
NewDashCount[indexA, "position"] <- indexA</pre>
 indexA <- 1 + indexA
} #removing gap rich columns
#
#Create a file with the position in the string that
should be kept
KeepColumns <- data.frame(NewDashCount[NewDashCount]
#Create a loop where the strings in the position about
together in the new file as a new set of strings.
NewAlignment <- data.frame("alignment" = character()</pre>
NewAlignment[1:nrow(Alignment), "alignment"] <- ""</pre>
indexB <- as.integer(1)</pre>
while (indexB <= nrow(NewDashCount)) {</pre>
 if(NewDashCount[indexB, "keep"]) {
  NewAlignment[ ,"alignment"] <-</pre>
```

```
str_c(NewAlignment[,"alignment"],
str_sub(Alignment[,"alignment"], indexB, indexB))
} #if we are keeping this column indexed by indexB
was flagged with "keep" <- TRUE
indexB <- 1 + indexB
}
#### Save the file
colnames(NewAlignment) <- "proteinA" #use proteinA of
needed
write.table(NewAlignment, file = "MutY_251.promals3)
= F, col.names = T, quote = F) #change title.</pre>
```

#### 4: Example of cutting the NTD of MUTYH

```
#Observe the new alignment with the gaps removed and
presence of the starting and ending residues of the
TempAlignment <- NewAlignment[ ,"alignment"]</pre>
#Write out the New Alignment file and make sure that
sequence is the structure sequence
write.table(NewAlignment[, "alignment"], file =
"MUTYH 250.promals3D", row.names = FALSE, quote = F
col.names = FALSE)
#Extract the last line of the alignment into a new :
last line is the structure sequence.
LastLine <- tail(TempAlignment, n=1)</pre>
#Find the sub string position of QASVS and GVANF
str_locate(LastLine, "QASVS") #Replace QASVS with st
sequence of domain A
str locate(LastLine, "GVANF") #Replace GVANF with en
sequence of domain A
#Cut the NTD from the beginning of the string to the
position.
MUTYH NTD <- data.frame(str sub(TempAlignment, 1 , 2</pre>
1 and 214 as indicated values from steps above
```

```
colnames(MUTYH_NTD) <- "proteinA"
# Write out the NTD domain sequences into a new file
write.table(MUTYH_NTD, file = "MUTYH_250_NTD.promals
row.names = FALSE, quote = FALSE)</pre>
```

#### 5: MI Calculator

Codes for the MI Calculator in the R program are avarequest to martin.horvath@utah.edu.

About the Author

Tara Tazehabadi UNIVERSITY OF UTAH

58. Pollinator

Influence on the

# Abundance and

# **Composition of**

# **Nectar Microbes in**

# Oenothera

# Macrocarpa

Eleanor Wachtel (University of Utah); Joshua Steffen (School of Biological Sciences, University of Utah); and Annika Kloepper

Faculty Mentor: Joshua Steffen (School of Biological Sciences, University of Utah)

Pollination is a crucial step in plant reproduction, essential for the cultivation of 80% of the 1400 crop plants grown around the world (1). Insects, specifically bees, play an integral role in the process by transferring pollen from one flower to another while taking advantage of energy sources, primarily in the carbohydrate rich nectar produced by flowers (2). These interactions between pollinators and energy sources are critical for effective pollination. Current research suggests that microbial communities in nectar can influence interactions between flowers and pollinators (3-5). Our research attempts to refine our understanding regarding the impact of foraging insects on microbial abundance and diversity in floral nectar.

We characterized microbial communities in Oenothera macrocarpa (evening primrose) because of its abundance of nectar, its day-long life span, and its large quantity of pollinating insect species. Our preliminary research identified several microbial species that colonize nectar. In order to see whether the pollinators visiting the O. macrocarpa species were depositing microbes, we limited pollination through bagging the flowers for their whole life cycle (from bud to wilted flower). We then extracted nectar from both the limited (bagged) and unlimited (open) flowers and cultured the microbes on two different media, LB and Nectar. The nectar media was used because of its ability to mimic the environment the microbes usually grow in, and the two different media types were used to foster non-redundant, differing growth of microbes. After a two-day incubation period, we counted the colonies grown on both media for both conditions (bagged and unbagged). We ran PCR, or polymerase chain reaction, on the colonies, using the conserved 16S region of the bacterial genome to identify the microbes. We found that open, pollinated flowers harbored a greater number of microbes

relative to flowers that insect visitation was prohibited (onesided, unpaired t-test, p-values < .05). Similarly, our data suggests a difference in the microbial diversity in nectar between bagged and unbagged flowers. A total of fifteen different taxonomic groups were identified from both the open and bagged floral nectar. However, in unbagged, pollinated flowers, eleven different taxa were identified while in bagged, unpollinated flowers only seven were identified. A number of taxa (i.e. Acinetobacter, Curtobacterium, and Enterobacterales) were only cultured from open flower nectar. These results generally align with previous research findings in alternative species and environments.

Our experiments confirm that foraging has a significant impact on the microbial diversity in nectar and suggests that this composition is directly related to the pollinators that visit this species. Further research will attempt to associate bacteria taxa to specific pollinator genera, in an effort to use the presence of particular microbial species to indicate and identify pollinator visitation in the absence of direct pollinator observation. Additionally, future work will focus on determining the impact of bacteria on chemical cues accompanying nectar and associated foraging preferences.

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DEPARTMENT XII

# College of Social & Behavioral Science
- 59. Neurocognitive
  - **Effects of Listing**
  - **Effort on Speech**
- **Perception in Older** 
  - **Adults: Evidence**
- from Event-Related

## **Brain Potentials**

Hope Caviness (Baylor University); Brennan Payne (Psychology, University of Utah); and Jack W. Silcox (University of Utah)

Faculty Mentor: Brennan Payne (Psychology, University of Utah)

#### Purpose

In older adults, there is a clear relationship between hearing loss and cognitive disruptions, including negative effects on speech comprehension. This leads adults to distancing themselves from participating in daily communicative activities. The goal of the current study is to use cognitive event-related brain potentials (ERPs) to understand higherlevel cognitive and brain functions as adults listen to perceptually demanding speech (e.g., in background noise).

### Method

We measured ERPs as older adults (N = 48, mean age = 71; range = 60 - 85) listened to sentences with expected and unexpected sentence-final words either in quiet or with background noise. We measured two cognitive ERPs time-locked to the sentence-final word: the N400 and the frontal negativity.

#### Results

We found that the N400 response, which indexes semantic processing, is delayed in background noise. This indicates that the early retrieval of words from long-term memory is hindered when listening effort is high. In addition, we observed a frontal negativity to expected words in quiet, which has been linked to high-level working memory related comprehension processes. This frontal negativity was eliminated in background noise. Thus, high-level comprehension processes are diminished when increased listening effort is required to overcome perceptual limitations.

#### Conclusion

This study demonstrates underlying differences in brain activity in older adults when processing auditory input in quiet compared to noise. Our findings demonstrate that older adults experience delays in word retrieval and reductions in highlevel comprehension processes when the listening environment is more perceptually demanding. Such findings may be clinically useful with respect to assessing hearing loss in parallel with cognitive functioning to improve the communicative experience of those who endure age-related hearing loss.

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60. Reflection on

## Undergraduate

### Research

### **Experience** -

## Lauren Christian

Lauren Christian (University of Utah)

Faculty Mentor: Sara Grineski (Sociology, University of Utah)

Of all of my college experiences, my undergraduate research experience had the greatest impact in informing my future academic and career aspirations. I served as an undergraduate on an air quality study during the 2021-22 academic year, and used the findings from that study to craft my honors thesis this semester. My research gave me a sense of direction and purpose, and made it clear to me that I wanted to work at the intersection of environmental justice and air quality issues. I have now secured an internship with NASA projecting the impact of dust from the Great Salt Lake on Utah's air pollution, and I have managed air quality projects as the Community Engaged Learning Coordinator for the SPARC Environmental Justice lab on campus. Neither of these positions would have been possible without my research background in air quality.

About the Author

Lauren Christian UNIVERSITY OF UTAH 61. Utilizing Feedback from Families with Asthmatic Children to Mitigate Air Pollution Health Consequences Lauren Christian (University of Utah)

Faculty Mentor: Sara Grineski (Environmental and Sustainability Studies, University of Utah)

Air pollution is an ongoing public health threat in the US and in Utah. While air pollution triggers asthma, we know little about what parents of asthmatic children think about what schools, health care providers and policymakers should

do to improve air quality and their children's health. Data to address this gap came from a 2021 study with twenty-seven families across Salt Lake County. Families were given low-cost indoor and outdoor air quality sensors and asked to observe connections between air pollution and their children's respiratory symptoms. Each parent completed a pre-interview, sixteen weeks of surveys, and a post- interview. This project draws on the post-interviews and is focused on changes that parents would like to see in how schools, healthcare providers, schools, and policy makers address outdoor and indoor air pollution and children's health. Of the 27 participants, 20 were non- Hispanic White, six were Hispanic, and one was African American. Five spoke Spanish as their primary language. The average age of the asthmatic children was 8.5 years. The first author coded the twenty-seven post-interview transcripts into parent nodes, i.e., "Schools," "Healthcare Providers" and Policymakers," which were then sub-coded into more specific categories. Parents requested that schools improve protocols to recognize and respond to asthma symptoms, improve indoor air quality, install air quality sensors, regulate PE and athletic events based on air pollution levels, and encourage anti-idling measures. In terms of healthcare, parents requested that their providers stay up-to-date on air quality research, screen patients for air pollution exposure, educate patients about connections between air pollution and asthma, and discuss air quality sensors and clean air resources with patients. Parents wanted policymakers to better monitor and regulate major sources of air pollution emissions; to expand public transportation, green spaces, and clean energy; and to work towards reduced healthcare costs while expanding resources for sensitive groups. Our research demonstrates that parents would like to see greater action from schools, healthcare

providers, and policymakers to reduce air pollution and the burden it poses to children's health. Our findings will be shared via informational pamphlets with these stakeholders to catalyze action on these issues.

About the Author

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# 62. Electric Bus Air

# **Quality Monitoring**

## **Platform: Findings**

### and Implications -

## Part 2

Jessica Cuello (University of Utah); Tabitha Benney (Political Science, University of Utah); Daniel Mendoza (Atmospheric Sciences, University of Utah; and Casey Olson (University of Utah)

Faculty Mentor: Tabitha Benney (Political Science, University of Utah)

During poor air episodes, the Salt Lake Valley (SLV) area suffers from an apparent pollutant heterogeneity that

disproportionately affects neighborhoods across the valley. The University of Utah, in partnership with the Utah Transit Authority (UTA), created a state-of-the- art mobile air quality monitoring network in 2014 that is uniquely equipped to diagnose the state of local air quality with an unprecedented level of detail. A vast mobile network of sensors becomes pivotal in pinpointing these discrepancies during peak events to ascertain the level of inequality and possible inadvertent sources of pollution, such as industrial infrastructure shortcomings. This study explores the recent expansion of the network to include research-grade sensors on UTA's electric bus fleet (eBus). In combination with the existing mobile sensor network, the eBus contributes to a new level of spatial/ temporal resolution and accuracy to the network. Increasing the scope of the network to include data with an unprecedented amount of control over its spatial coverage has improved the accuracy of inverse distance interpolation methods. Our preliminary data analysis has revealed severe pollution hotspots on a neighborhood scale during peak events that would not have been picked up by stationary sensors or the existing TRAX network. Based on early results, these invaluable data will be a launching ground for a series of studies that improve our understanding of environmental justice issues and the behavior of air pollutants in the SLV.

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# 63. Physiological

## Responses to an

# **Online Infant Cry**

# Stimulus in

## **Expectant Mothers**

Shane Denherder (University of Utah); Dylan Neff (University of Utah); Joshua Marchant (University of Utah); Rose McLaughlin (University of Utah); K. Lee Raby (Psychology, University of Utah); Sheila E. Crowell (Psychology, University of Utah); and Elisabeth Conradt (Psychology, University of Utah) Faculty Mentor: Elisabeth Conradt (Psychology, University of Utah)

The COVID-19 pandemic has introduced new challenges for keeping participants and research assistants safe during laboratory visits. Many researchers altered their protocols in novel ways– for example, to an online platform– to adapt to the pandemic. The present study compares the physiological effects of an online adaptation of an infant cry stimulus to the traditional laboratory-based cry task. Video and audio recordings of an infant cry are commonly used by developmental studies to evoke and measure sympathetic and parasympathetic nervous system responses to this attachmentrelevant stressor in pregnant women.

Measuring respiratory sinus arrhythmia (RSA) and electrodermal activity (EDA) during infant cry presentation, we analyzed a unique sample (N = 115) of pregnant women in their third trimester, half of whom observed the infant cry stimulus in the laboratory before the pandemic. Results revealed that EDA increased, and RSA decreased as expected and that setting– online

versus laboratory– had no effect on RSA or EDA responses to stimulus. These results demonstrate the ability of remote tasks to elicit an attachment-relevant stress response in pregnant women for remote data collection. Implications include the possibility of these data being collected in more ethnically and geographically diverse populations of pregnant people, including rural and marginalized populations unable to travel to large research facilities.

Note: This is the abstract from the in-progress manuscript that will be submitted to a peer-review journal. Journal requirements stipulate that submissions may not be previously published elsewhere, the abstract and references are provided for the Undergraduate Research Journal.

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## 64. **Reflection on**

## Undergraduate

### Research

### **Experience - Marin**

## Macfarlane

Marin Macfarlane (University of Utah)

Graduate Student Mentor: Amy McDonnell (Psychology, University of Utah)

Through this opportunity, I have gained a deeper appreciation of research and its ability to expand our knowledge and induce change in the world. As a future psychologist, I hope to study various aspects of neurophysiology, so I have been extremely fortunate to use brain-imaging technology, such as electoencepholography (EEG), as an undergrad. I believe this will only make me a stronger candidate for graduate school. I feel that this experience has given me the confidence I need to pursue my goal of becoming a clinical psychologist. Working alongside a mentor has also been extremely beneficial, as Amy has motivated and inspired me to apply to Ph.D. programs and achieve my goals.

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# 65. Effects of

### Natural and Urban

### Imagery on

## **Error-Related**

### Negativity

Marin Macfarlane (University of Utah); Amy McDonnell (University of Utah); and David Strayer (Psychology, University of Utah)

Graduate Student Mentor: Amy S. McDonnell (University of Utah)

#### Abstract

Attention Restoration Theory (ART) proposes that urban environments deplete our attentional resources and natural environments counteract this depletion by allowing our attentional system to rest and recuperate (Kaplan, 1995).

behavioral research supports attention-related Previous benefits of both immersion in nature and viewing images of nature, but little research has utilized brain-imaging to investigate the neural mechanisms underlying these benefits. In the present study, we use electroencephalography (EEG) to investigate the effects of viewing nature imagery in comparison to urban imagery and no imagery on the Error-Related Negativity (ERN), a component of the Event-Related Potential (ERP) related to cognitive control and attention. Previous research has shown an increase in ERN amplitude during immersion in nature compared to immersion in an urban environment, indicative of an increase in cognitive control capacity during immersion in nature. We similarly measured amplitude of the ERN after participants viewed either nature or urban imagery to see if images of nature would have the same effect as immersion in nature does. We predicted an increase in the ERN amplitude for the nature imagery condition compared to the urban imagery condition as well as a no imagery control. However, we found no statistically significant difference in ERN amplitude between the nature and urban imagery conditions, as well as between the nature and no imagery conditions, suggesting that viewing nature imagery may not have the same effect on brain activity as immersion in nature. Future research could investigate whether viewing nature imagery for longer periods of time may be necessary to significantly influence the ERN.

#### Introduction

In modern society, it is increasingly common for people to spend little time outdoors. Although 75% of American adults value spending time in nature, over 50% spend less than 5 hours outside per week (Kellert et al., 2017). American parents

of children ages 8 to 10 report that their children spend 3 times as many hours indoors watching television or playing computer games than they do playing outside (Kellert et al., 2017). Significant barriers to spending time in nature include the allure of technology, indoor obligations such as school or work, and a lack of accessibility to natural environments. By 2050, 70% of Americans are predicted to live in urban environments, making accessibility to natural environments increasingly difficult (Heilig, 2012). These statistics are problematic because certain behavioral and psychological benefits may become compromised due to a lack of access and time in natural environments. Spending time in nature has been shown to reduce depressive symptoms and improve mood (Gidlow et al., 2016), as well as improve attention and performance on various cognitive tasks (Berman et al., 2008; Hartig et al. 2003; Ohly et al., 2016).

Attention is a valuable resource required for successful completion of various cognitive tasks; however, attention is a limited resource and is therefore subject to depletion by attentionally demanding tasks (Baumeister et al., 2018). Attention Restoration Theory (ART) proposes that urban environments, in particular, deplete our attentional resources and that natural environments can counteract this depletion by allowing our attentional system to rest and recuperate (Kaplan, 1995). Numerous studies have supported this theory and shown positive behavioral effects following nature exposure, such as improved cognitive performance on tasks requiring creativity (Atchley, Strayer, & Atchley, 2012), sustained attention (Berman et al., 2008; Hartig et al., 2003), and working memory (Bratman et al., 2015). Although those who have direct, physical immersion in nature typically receive the most benefit (Townsend & Weerasuriya, 2010), there is evidence suggesting that simply viewing images of nature can induce behavioral benefits related to attention (Berman et al., 2008). A study by Berto (2005) found that those who completed an attentionally demanding task, viewed nature imagery, and then completed the task again performed significantly better the second time than those who did not view nature imagery between tasks. Moreover, Ulrich (1979) found that students who viewed nature imagery prior to taking a test reported significantly lower stress levels and greater levels of positive affect than those who did not. Nature imagery has also been shown to reduce anxiety, stress, and irritability in a variety of applied environments, such as hospitals, dementia care facilities, and prisons (Ulrich & Nadkarni, 2009; Bratman et al., 2012; Nadkarni et al., 2017).

Research on the behavioral effects of nature exposure has been accumulating for decades, but little research has utilized brain-imaging investigate neurophysiological to the mechanisms underlying these reported behavioral effects. Bratman et al. (2015) employed fMRI and found reduced activity in the subgenual prefrontal cortex (sgPFC), a brain area associated with depression, after participants completed a nature walk as compared to participants who completed an urban walk. Van Praag et al. (2017) found fMRI results in alignment with ART: participants who listened to nature sounds as compared to urban sounds had increased activity in the default mode network (DMN), which indicates decreased AN activity (Buckner et al., 2008).

In addition these initial studies exploring to neurophysiological responses to nature as measured with fMRI, preliminary evidence research has found of electrophysiological brain responses to nature, measured with EEG (e.g. Aspinall et al., 2015; LoTemplio et al., 2020; McDonnell et al., under review; Scott et al., in prep; Ulrich,

1981). Ulrich (1981) found greater alpha wave amplitude while participants viewed nature imagery as compared to urban imagery, suggesting higher relaxation, alertness, and lower arousal. Another study found that participants taking a nature walk, as compared to participants taking an urban walk, had neurophysiological signals that suggested a meditative state, along with decreased frustration and arousal (Aspinall et al., 2015). Important to the present study, LoTemplio et al. (2020) found an in error- related negativity (ERN) increase amplitude during a 5-day nature trip as compared to pre- and post-trip recordings that took place in an urban environment. The error-related negativity is an event- related potential (ERP) described as a negative deflection in the brain waveform occurring within 100 ms after an individual makes an error on a task. The amplitude of the ERN increases (becomes more negative) as motivation to avoid errors on a task increase (Gehring et al., 1993; Hajcak & Foti, 2008), and it is thought to be positively correlated with cognitive control and indicative of AN activity-such that as attention allocated to the task increases, ERN amplitude increases. The ERN also increases when individuals are rewarded for good performance (Hajcak et al., 2005), and is positively correlated with levels of anxiety (Endrass et al., 2008; Gehring et al., 2000; Hajcak et al., 2003).

LoTemplio et al. (2020) initially hypothesized that there would

be a decrease in ERN amplitude when performing a cognitive task during a 5-day camping trip compared to pre- and posttrip recordings, as ERN is thought to be a manifestation of AN activity. Since ART predicts that our AN rests and downregulates in nature, and previous studies have shown increased ERN is related to anxiety, the ERN was thus predicted to decrease for the nature condition. This prediction aligned with the results of their pilot study, but their follow-up study found a significant increase in ERN amplitude for the nature condition compared to urban conditions, appearing to contradict ART. However, there are potential explanations for this discrepancy. Their pilot study was underpowered, which can lead to less stable results (Button et al., 2013; Szucs & Ioannidis, 2017); additionally, under the framework of ART, it is possible that sufficient time had already been spent in nature and, therefore, cognitive resources were replenished and ready for use at the time of the task (Kaplan, 1995), leading to more attentional resources and thus an increase in ERN amplitude. Large ERN amplitudes are also associated with several abilities necessary for strong performance on cognitive tasks, such as increased working memory capacity (Coleman et al., 2018), desire for accuracy (Gehring et al., 1993), and self-regulation (Legault & Inzlicht, 2013; Potts et al., 2006). LoTemplio et al. (2020) provided compelling evidence in their follow-up study for the effects of nature on ERN amplitude. Because these results were unexpected based on the pilot data, further research needs to be conducted to clarify the effects of nature on human neurophysiology.

In order to further explore the neural processes behind ART and the effects of different types of nature (i.e., immersion compared to viewing images), the present study measures the effects of viewing nature imagery compared to urban imagery on error-related negativity (ERN) amplitude. Based on the results of LoTemplio and colleagues (2020), we hypothesize that ERN amplitude would increase after viewing nature imagery as compared to viewing no imagery or urban imagery, indicating greater cognitive and attentional resources available to allocate to the task. This research will provide further implications about our relationship to nature and how it affects us on a neurophysiological level.

#### Method

#### Participants

Participants (N=56) comprised of both University of Utah undergraduate students as well as community volunteers from the greater Salt Lake City area. Participants were selected using convenience sampling through SONA, a participant pool management system, and through flyers

posted around campus as well as throughout the surrounding area. 77% of participants identified as White/Non-Hispanic, 17% identified as Asian, 4% identified as Hispanic/Latino, and 2% identified as Black/African American. Participants were between the ages of 18 and 50 (M = 25.13, SD = 5.46). Participants were compensated with earning either course credits through SONA, or a payment of \$70 cash.

#### Materials

The nature and urban images used in this study were obtained from a past study investigating the effects of nature imagery on behavior (Berman et al., 2008). Participants' ERN amplitudes were measured using BioPac Systems EEG electrodes while completing the Eriksen and Eriksen (1974) Flanker task, a cognitive task designed to elicit the ERN. In the Flanker task, participants are asked to respond to the central letter in a five-letter sequence of either congruent stimuli, which consists of all identical letters (e.g., SSSSS or HHHHH), or incongruent stimuli, which has a middle letter associated with the opposite response (e.g., SSHSS or HHSHH). The ERN is generated on each trial that the participant makes an error. EEG data was viewed online through the data collection and analysis software AcqKnowledge.

#### Procedure

Participants came into the lab for three 2-hour testing sessions spaced one week apart. During the EEG setup, participants filled out a series of self-report questionnaires for a different study that will not be reported in the current study. Participants were randomly assigned to either the nature imagery condition or the urban imagery condition, with 28 participants in each condition. Following EEG setup. participants were taken to an outside patio on campus where they completed two cognitive tasks for a different study, followed by the Eriksen and Eriksen (1974) Flanker task used for the current study. Participants completed three testing sessions, with the imagery manipulation at testing session two, consistent with the design of LoTemplio et al. (2020). All three testing sessions were identical, except for the start of the second session, where participants viewed either 10 minutes of nature imagery or urban imagery before completing the Flanker task. During session one and session three, participants were not provided an imagery stimulus, and sat for 10 minutes in front of a concrete wall before beginning any cognitive tasks. Following each testing session, participants returned to the lab to fill out a final self-report questionnaire unrelated to the present study.

#### EEG Recording and Processing

We used BioPac Systems electrodes to record EEG, placing electrodes at Cz, Fz, and Pz, and additional electrodes on participants' faces to record electrooculographic (EOG) signals such as blinks and eye movements for later data cleaning. We placed a reference electrode behind the right ear on the mastoid bone and a ground electrode in the middle of the forehead.

EEG data was processed in MatLab with the EEGLab and ERPLab toolboxes (Lopez- Calderon & Luck, 2014). We first downsampled the data to 250 Hz and filtered from 0.1-30 Hz. Next, we identified all the blinks and eye-movements in the data and corrected them using eye- movement correction procedure (EMCP; Gratton et al., 1983). We then identified the timepoints in which a participant made either a correct response or an incorrect response and averaged them together separately to be left with a single waveform of correct responses and another waveform of incorrect responses. We then subtracted the correct brain activity from the incorrect brain activity to be left with the ERN (which is a difference wave of incorrect minus correct).

#### Analysis Plan

For our first analysis, comparing the effects of viewing nature imagery and no imagery from Session 1 to 2 to 3 on the ERN, we used a linear mixed effects model to control for repeated measures within an individual and for missing data. In our mixed model, Participant ID was included as a random intercept, ERN amplitude was our dependent variable, and Session was our independent variable, which we included as a fixed effect. For our second analysis comparing the effects of viewing nature imagery and urban imagery on ERN amplitude just at Session 2, we used a Welch Two Sample t-Test.

### Results

To test the hypothesis that the ERN increases after viewing nature imagery as compared to no

imagery, we analyzed ERN amplitude within the nature viewing participants at Session 1 (M=-3.01, SD=2.86), Session 2 (M=-2.79, SD=2.21), and Session 3 (M=-2.72, SD=2.94). The mean amplitudes of the ERN during no imagery (Session 1 and Session 3) and nature imagery (Session 2) are shown in Figure 1. We ran a linear mixed effects model to determine whether the change in ERN amplitude between sessions was statistically significant. The ERN waveforms for Sessions 1, 2, and 3 are shown in Figure 2. There was not a statistically significant change from Session 1 to Session 2 (p=.719), from Session 1 to Session 3 (p=.488), or from Session 2 to Session 3 (p=.728). To test the hypothesis that the ERN increases after viewing nature imagery (M=-2.79, SD=-2.21) as compared to viewing urban imagery (M=-3.84, SD=2.83), we analyzed ERN amplitude between the nature and urban conditions at Session 2. The mean amplitudes of the ERN during nature and urban imagery conditions are shown in Figure 3. We ran a Welch Two Sample t-test to determine whether the change in ERN amplitude between nature and urban imagery conditions was statistically significant. The ERN waveforms for the nature and urban imagery conditions at session 2 are shown in Figure 4. There was no statistically significant difference between ERN amplitudes of the nature condition and the urban condition (t(50.85)=1.548, p=.128). Therefore,

both hypotheses in the current study were not supported.



Fig. 1. The mean amplitudes of the error-related negativity (ERN) during no imagery (Session 1 and Session 3) and nature imagery (Session 2) conditions at electrode cite Cz. This shows data from only the nature viewing participants (N=28).



Fig 2. The error-related negativity (ERN) waveform during no imagery (Session 1 and Session 3) and nature imagery (Session

2) conditions at electrode site Cz, where 0 ms is the response onset and the ERN is the negative deflection observed between 0-100ms after response.



Fig 3. The mean amplitude of the error-related negativity (ERN) during the nature imagery and urban imagery conditions (Session 2) at electrode site Cz.



Fig 4. The error-related negativity waveform during the nature imagery and urban imagery conditions (session 2) at electrode

site Cz, where 0ms is the response onset and the ERN is the negative deflection observed between 0-100ms after response.

#### Discussion

The present study investigated the effects of viewing nature imagery, as compared to viewing no imagery or urban imagery, on the ERN amplitude. Previous research has suggested that large ERN amplitudes are associated with greater general cognitive ability (Larson & Clayson, 2011), working memory capacity (Coleman et al., 2018), and motivation to perform tasks well (Boksem et al., 2006). Since nature exposure has been shown to increase performance on various cognitive tasks, particularly those that demand working memory capacity and cognitive control (Ohly et al., 2016), we anticipated that viewing nature imagery prior to completing the Flanker task would increase the ERN amplitude generated by the task. However, our findings did not support this hypothesis, as there was no statistically significant change in ERN amplitude between the nature and no imagery conditions, as well as between the nature and urban imagery conditions.

The findings of the current study suggest that viewing nature imagery has no effect on the ERN. Our null results differ from LoTemplio et al. (2020), who found a statistically significant increase in ERN amplitude during an immersive nature exposure. These divergent findings suggest that nature imagery may not be as powerful as immersion in nature as it relates to influencing the ERN. Although there is evidence to support that direct immersion in nature and indirect exposure to nature, such as viewing nature imagery, have similar behavioral effects, some evidence suggests that direct immersion in nature provides greater benefits (Kaplan, 1993; Kahn et al., 2009). It may be that the neurophysiological effects of nature immersion and viewing nature imagery are also different in this way; that is-the neurophysiological effects of nature immersion are more powerful than the those of indirect exposure to nature. This could contribute to why we did not find a significant change in ERN amplitude for the nature imagery condition.

### Limitations & future directions

It is possible that viewing nature imagery for longer periods of time may be necessary to significantly influence the ERN. Based on findings from LoTemplio et al. (2020), the AN may have needed more time to downregulate during nature imagery exposure so it could achieve restoration and therefore greater ERN levels by the time of the Flanker task. At present, the time-course of the behavioral and neural effects of nature is poorly understood. It may be possible that the amount of time needed for nature exposure to induce changes in the ERN is longer than the timeframe used in the current study (i.e., 10 minutes of imagery viewing). Future studies may want to increase the amount of time individuals are exposed to imagery stimulus and compare the effects of different viewing durations on the ERN. There may also be individual differences in the amount of viewing time required to induce changes in the ERN, which we were unable to investigate in the current study; therefore, future research may want to collect enough data to be sufficiently powered for individual difference analyses.

Although the current study did not find statistically significant changes in the ERN as a result of viewing nature imagery, previous work has documented changes in the ERN during nature immersion (LoTemplio et al., 2020). Because of these contrasting findings, it is possible that the benefits of viewing nature imagery may not have the same neural mechanisms as immersion in nature. Further research is needed to clarify the neurophysiological effects of nature exposure, and to determine whether the benefits of nature immersion and viewing nature imagery have similar neural mechanisms. Continuing such research could be an important factor in influencing public policy to protect our natural environments, as they are increasingly threatened by climate change, industrialization, and urbanization. Regarding specifically the nature imagery research, it is important to develop our understanding of its neurophysiological effects as it may have the potential to provide our increasingly urbanized world with greater accessibility to the psychological benefits of nature exposure.

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**Social Science:** 

Intersectional

**Praxis and the Role** 

of

**Community-Based** 

**Research-Practice** 

## **Partnerships**

Tara Jill Olson (University of Utah)

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

#### Introduction

In 2020 a worldwide health crisis and parallel social movement illuminated the existing social inequalities that have only become exacerbated by the ongoing global pandemic. Within academia and research, the pandemic has triggered a broader conversation about the lineage of intersectionality as both a lived reality and critical concept in intellectual work. Intersectionality describes the confluence of multiple categories of identity that interact and intersect each other along the dimension of race, class, gender, sexuality, and (dis)ability. At its core, intersectionality suggests that singleaxis approaches (e.g., analyzing race alone or analyzing gender alone) cannot adequately address the lived experience of the individual, particularly for multiply-stigmatized persons. Only recently have researchers begun to consider intersectionality in their theories yet research agendas continue to lack critical investigation into the repressive mechanisms that uphold systems of oppression and how best to research marginalized communities without inflicting additional harm knowing that our institutions of disenfranchisement have been built in some ways from knowledge generated from the ivory tower of academia.

#### Purpose

This project responds to the need to thoughtfully integrate intersectionality within research methodologies and measurements within the field of social science through intentional community engagement and incremental institutional change focused on the transformative goal of eradicating inequality.

#### **Methods: Literature Review**

The aim of this project was to evaluate the impact of intersectional praxis in research by studying previous research agendas that had implemented ethical research-practice partnerships, specifically the partnerships with communities that have been disenfranchised by institutions that support the power of the elite. This involved investigating and analyzing different approaches to the study of marginalized communities that worked to mitigate some of the negative aspects of community-based research by using social categories meaningfully in order to challenge previous single-axis research methods. Foundational to our study was the community-based research project conducted by Miewald et al. in researching foodscapes in collaboration with the houseless community in Ontario, Canada. The work of Miewald and the investigative team helped to provide evidence for this project by providing an example of a community-based research project that was intentionally conducted to avoid further alienating or stigmatizing a marginalized population by creating a bidirectional relationship between researchers and participants.

#### Results

From our research, we outline the following as important values for conducting ethical community-based research projects:

- The investigative team works in collaboration with a community advisory board.
- Paid peer research associates (PRA's), each of whom has life experiences in common with the study population, are hired as part of the research team.
- PRAs and other members of the community are involved in various aspects of the study including the research design, administration, interpretation, and dissemination of results. The importance of involving those from the community with similar life experiences to the population being studied

helps to break down traditional divisions between academic researchers, subjects, and the wider community, allowing for a greater diversity of perspectives considered in the research design and measurement.

#### Conclusion

Effective research-practice partnerships establish the integration of members of the community as well as community organizations and organizers as co-collaborators in the knowledge-generating process while measuring and analyzing experiences in intersectional ways. From our research, we determined that a successful community-based research project involves members of the community in all areas of the study. This project challenges previous research methodologies focused on single-axis issues by working from an intersectional praxis. The project has important implications for social justice and future research by highlighting the possibility of change and structural progress through community-based research that better explains the experiences of all people, especially those individuals being impacted the most by inequitable systems.

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# 67. Reflection on

## Undergraduate

## Research

## **Experience - Alicia**

# Quick

Alicia Quick (University of Utah)

Graduate Student Mentor: Amy McDonnell (Psychology, University of Utah)

My undergraduate research experience has been extremely beneficial and a huge learning experience. I was fortunate enough to work in a great lab with a very involved mentor. I got to experience the various parts of research from collecting data, analyzing data, and putting the findings into a paper. With all the things I learned I feel more confident in my understanding of how research works and have acquired many skills I otherwise wouldn't have. The impact of this on my education has been that I was able to finish my Human Factors Certificate final project doing research I found interesting. I also have strengthened my writing skills as well as my experience working with real data. My future goals have been impacted by opening up more opportunities to work as a human factors researcher by giving me experience that will make me a more competitive candidate in that job market. I'm truly grateful for the opportunity to have worked in this lab and with this research.

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## 68. Effects of

## Natural and Urban

## Imagery on the

## **Error-Related**

## Negativity

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Much research has gone into studying the benefits of exposure to natural environments, especially benefits related to attention, stress, and mood. However, there is an open question related to whether an individual must be exposed to real nature to receive these benefits, or if viewing images of nature has the same power. The present study uses electroencephalography (EEG) to explore the effect of viewing nature imagery on brain activity related to attention. Specifically, it measures the amplitude of the error-related negativity (ERN), a brainwave that indexes attentional control, after viewing images of nature. Building upon prior literature that suggests an improvement in attention (as indexed by an increase in amplitude of the ERN) during immersion in real nature, we predicted a positive relationship between viewing nature imagery and ERN amplitude, such that ERN amplitude would increase as a result of viewing nature images compared to urban images or a noimage control. However, data from 56 participants revealed that there was no influence of viewing nature imagery on the amplitude of the ERN, suggesting that there are characteristics of immersion in real nature that influence brainwaves related to attention that aren't present in just pictures of nature.

#### Introduction

The theory that attention is a limited resource is widely accepted. For example, when we are forced to exert effortful attention for long periods of time, our attentional resources can become depleted, decreased attentional performance leading to (Baumeister, Bratslavksy, Muraven, & Tice, 1998). This limitation of attention affects things such as what we can give our attention to, how well we can perceive and process information, and how efficient we are when performing various tasks (Oberauer, 2019). Knowing this, studying how to restore our easily depleted attentional resources is important. Attention Restoration Theory (ART; Kaplan, 1995) proposes one way to restore attention-spending time in nature. ART argues that natural environments contain less attentional demands, allowing us space to recuperate our attentional

resources, leading to increased attentional performance. Since many people are spending less and less time in nature and more time in urban environments, understanding the significance of nature's restorative effects could change how we prioritize spending time in nature.

Many behavioral studies have explored the ideas set forth by Attention Restoration Theory (ART), which proposes that exposure to nature can "restore" various aspects of attention that are depleted by our everyday, urban environments (Kaplan, 1995). These studies have shown improved performance on cognitive behavioral tasks requiring creativity (Atchley, Strayer, & Atchley, 2012), sustained attention (Berman, Jonides, & Kaplan, 2008), and working memory (Bratman et al., 2015) after nature exposure. In general, the findings of these studies support ART's proposition that exposure to nature positively affects our attentional performance.

However, showing nature's restorative effects using simple behavioral metrics is one way to study ART. Using neurophysiological metrics adds information about the actual changes that are happening in the brain when people interact with nature and how it later influences their behavior. Thus, using neurophysiology allows for a deeper understanding of nature's influence on the individual from multiple levels of analysis. Less research has investigated the neurophysiological mechanisms underlying the behavioral benefits of time in nature. One study run by Bratman et al. (2015) employed fMRI before and after a 50-minute walk and found reduced activity in the subgenual prefrontal cortex, an area in the brain associated with depression, in participants who completed a nature walk as opposed to an urban walk. Other preliminary research has found evidence of attention-related electrophysiological brain responses (using EEG) to nature (Aspinall et al., 2015; LoTemplio et al., 2020; McDonnell et al., under review; Scott et al., in prep). These findings are important in helping us better understand nature's restorative properties on a neural level as opposed to just a behavioral level.

The present study builds off a prior study conducted at the University of Utah. In this study, LoTemplio and colleagues (2020) took participants on a 4-day camping trip and used EEG to measure brain activity related to attention before, during, and after the exposure to nature. In particular, they measured changes in the error-related negativity (ERN), a brain component related to attentional control. The ERN is a negative-deflection in frontally- and centrally distributed brainwaves (electrodes Fz and Cz) that appears within 100ms after an individual makes an error on a task. The ERN is not present when an individual makes a correct response, so it is thought to index our ability to recognize an error and allocate our

attention accordingly to correct subsequent behavior (Gehring et al., 1993). The ERN is most often generated by the Flanker Task (Eriksen & Eriksen, 1974), so participants in this study completed a Flanker Task before, during, and after the camping trip in order to elicit the ERN. LoTemplio and colleagues (2020) found that ERN amplitude increased during the nature exposure, thought to represent an increase in attentional control abilities in nature and thus potentially indicative of attention restoration (LoTemplio et al., 2020). The present study serves as the control study to LoTemplio et al. (2020) and seeks to answer whether or not someone needs to be fully immersed in nature to receive attentional benefits. or if viewing nature images is enough to improve attentional control. In this study, we measured the same ERN brainwave elicited by the Flanker Task to see if it acted similarly to what was found in the nature immersion study.

As mentioned, our study utilizes electroencephalography (EEG) to measure changes in the amplitude of the error-related negativity (ERN). The ERN peaks within 100 milliseconds after an individual makes an error on a task. The ability to recognize the error is related to the attention allocated to the task. Studies have shown that when someone is more motivated to avoid errors on a task, the ERN increases (Gehring et al., 1993; Hajcak & Foti, 2008). A larger ERN is also associated with better self-regulation (Legault & Inzlicht, 2013; Potts et al., 2006) and increased working memory capacity (Coleman et al., 2018). The use of EEG allows us to explore whether the ERN is influenced by exposure to nature imagery. This may provide further evidence of Attention Restoration Theory beyond just behavioral metrics, as well as answer the question of whether simply viewing images of nature is enough to see the restorative changes proposed by ART (as opposed to being immersed in real nature).

We hypothesized that if nature images are enough exposure to nature to elicit attentional changes in the brain, there will be an increase in the amplitude of the ERN after viewing 10 minutes of nature imagery, which would conceptually replicate the results of LoTemplio et al. (2020). On the other hand, if images of nature are not enough exposure to restore attention there will be no change in the amplitude of the ERN across the three testing sessions. This would suggest that there is something unique about immersion in nature as opposed to just viewing images of nature when it comes to restoring attentional resources.

#### Method

Participants

Participants (N=56) were recruited from the greater Salt Lake area and paid \$70 compensation at the end of the study. The sample was compromised of 41 females (73%) and 15 males (27%). The participants ages ranged from 18-50 (M = 25.13, SD = 5.46). The majority of the sample identified as White/ Caucasian at 77% with 17% identifying as Asian, 4% identifying as Hispanic/Latino, and 2% identifying as Black/African American.

Materials

EEG data were recorded using the BIOPAC EEG system (BIOPAC Systems, Goleta, CA, USA) and reusable electrodes (NATUS Neurology Grass gold-surface electrodes, model F-E5GH-48) and observed through the AcqKnowledge (Version 5.0) software while participants were testing.

To elicit the ERN (our dependent variable), participants completed 800 trials of the Flanker Task (Eriksen & Eriksen, 1974) while the EEG was recorded, consistent with LoTemplio et al. (2020). The Flanker Task asked participants to respond to the central letter in a five-letter sequence. There were either congruent stimuli consisting of all identical letters (e.g., SSSSS or HHHHH), or incongruent stimuli (e.g., SSHSS or HHSHH). Participants were instructed to respond quickly and accurately to the central letter using keys on the keyboard. Importantly, the ERN is generated on each trial that the participant makes an error on this task (e.g., when they respond that the middle letter is an H when it is actually an S).

The nature and urban images that were utilized in this study were the same images used by Berman, Jonides, and Kaplan (2008) in a study that found attentional benefits associated with viewing nature compared to urban imagery. The nature and urban images were presented to the participant via a 10-minute PowerPoint slideshow before they completed the Flanker Task.

EEG Data Processing

EEG data was processed in MatLab using the EEGLab and ERPLab toolboxes (Lopez- Calderon & Luck, 2014). The data was first down sampled to 250 Hz and filtered from 0.1-30 Hz in order to only capture brain activity and not surrounding electrical activity from the environment. We then identified all the blinks and eye-movements in the data and corrected them using eye-movement correction procedure (Gratton, Coles, & Donchin, 1983). Then, once the data was clean of eye-movements, we identified all the timepoints in which a participant made a correct response and all the timepoints in which they made an incorrect response and then averaged them together separately to be left with a single waveform of correct responses and a single waveform of incorrect responses. We then subtracted the correct brain activity from the incorrect brain activity to be left with the ERN (which is technically a difference wave of incorrect minus correct).

#### Design

Consistent with LoTemplio et al. (2020), participants completed three testing sessions, spanning three weeks, as the within-subjects component. However, in the present study at Session 2, half the participants were randomized to view nature images and half viewed urban images, making Image Type a between-subjects component. This resulted in a mixed design with both a within- and between-subjects component. At Sessions 1 and 3, participants looked at a concrete wall for 10 minutes before completing the Flanker Task. At Session 2, they viewed environmental imagery (nature or urban) for 10 minutes before completing the Flanker Task. Thus, like LoTemplio et al. (2020), the environmental manipulation occurred at Session 2.

#### Procedures

At each session participants read through and signed a consent document and had questions answered by the researcher. Researchers then explained the study set up and what the participant could expect at each session. Participants were then set up with the EEG system in the lab while filling out some self-report questionnaires for a different study. Scalp electrodes were placed at Fz, Cz, and Pz with a reference electrode on the right mastoid bone, a ground electrode on the middle of the forehead, and eye electrodes above and below the right eye for recording blink activity.

During the first session all participants spent the first 10 min staring at a concrete wall outside of the BEHS building on the University of Utah campus. They did this in a weatherproof enclosure sitting in a chair to protect the EEG equipment from the elements. After those 10 min they completed the Flanker Task to elicit the ERN. At the second session they completed the same procedure, but instead of staring at the wall for 10 min they either viewed urban images for 10 min or images of nature for 10 min (Berman et al., 2008) before completing the Flanker Task. Participants were randomly assigned to view either urban (N=28) or nature images (N=28) making the study both between subjects (urban vs. nature) and within subjects (no imagery vs. imagery). The third testing session looked identical to the first, in which the 10 mins were spent staring at the concrete wall. Thus, the second testing session contained the nature manipulation (similar to LoTemplio et al., 2020). At the end of each session, they filled out a post-task mindfulness survey for a different study while the EEG electrodes were removed. At the end of the third session participants were compensated and debriefed.

#### Results

This research was aimed at examining the relationship between ERN amplitude and viewing nature imagery. We started by analyzing the data to get descriptive information including participant's age, gender, and ethnicity (reported above). We then processed the ERN data for each participant in MatLab using the procedures outlined in the Methods section. We conducted two separate analyses to compare ERN amplitude between nature imagery and no- imagery (by viewing the results of the nature imagery participants at sessions 1, 2, and 3) and to compare ERN amplitude between nature imagery and urban imagery only at Session 2.

To analyze the ERN data for the nature imagery viewing participants from Session 1 to 2 to 3, we used a linear mixed effects model using the lme4 package (version 1.1-17) in the R

language for statistical computing (version 1.1.442) to account for individual differences and missing data. We found that there was no statistically significant change in ERN amplitude for

the nature imagery viewing participants from Session 1 to Session 2 (p = 0.719). In addition, there was no statistically significant difference found in ERN amplitude from Session 1 to 3 (p =

0.488) or from Session 2 to Session 3 (p = 0.728).



Figure 1. ERN amplitude difference wave for nature imagery sessions 1, 2, and 3.



Figure 2. Mean amplitude of the ERN for nature imagery sessions 1, 2, and 3.

To compare ERN amplitude at Session 2 between the nature viewing participants and the urban viewing participants, we ran an independent samples t-test. We found that there was no

significant difference in ERN amplitude between the two groups (t(50.85)= 1.55, p=.128).



Figure 3. ERN amplitude difference wave for nature vs. urban imagery session 2.



Session 2

Figure 4. Mean amplitude of the ERN for nature vs. urban imagery.

#### Discussion

In this study we looked at the ideas set forth by Attention Restoration Theory (ART) which proposes that time in natural settings restores our attentional resources (Kaplan, 1995). A previous study by LoTemplio and colleagues (2020) tested ART by taking participants out into nature on a 4-day camping trip and measuring their ERN using EEG equipment while performing a standardized Flanker Task (Eriksen & Eriksen, 1974). They found a significant increase in ERN amplitude when participants were on the camping trip compared to before and after, suggesting better attentional control during immersion in nature. We ran the present study to compare the findings of LoTemplio et al. (2020) to the ERN amplitude of participants who viewed images of nature as opposed to spending time in a natural environment. Our hypothesis was that if nature images are enough exposure to nature to elicit attentional changes in the brain, there would be an increase in the amplitude of the ERN after viewing 10 minutes of nature imagery. On the other hand, if images of nature are not enough exposure to restore attention, there would be no change in the amplitude of the ERN across the three testing sessions, suggesting that there is something unique about immersion in nature as opposed to just viewing images of nature when it comes to restoring attentional resources.

The results of the study found that there was no statistically significant difference in amplitude of the ERN after viewing nature imagery compared to no imagery, nor after viewing nature imagery compared to urban imagery. These findings are informative in that they show the importance of immersing oneself in nature as opposed to just viewing pictures of nature when it comes to restoring attentional resources. Since previous studies have shown the positive effect of immersion in nature on attention restoration (Aspinall et al., 2015; Bratman et al., 2015; LoTemplio et al., 2020; McDonnell et al., under review; Scott et al., in prep), it's worth thinking about why viewing images of nature didn't produce the same results.

ART focuses on four components that lead to attention restoration: soft fascination, being away, extent, and compatibility (Kaplan, 1995). Soft fascination causes a decreased need for directed attention and opportunities for reflection which helps restore depleted attentional resources. Being away refers to when someone is free of the mental effort required for directed attention. Extent means the environment must be engaging enough for the mind, allowing for significant depth of processing. Compatibility indicates that the natural environment someone is in must be compatible with them. For example, someone who has a strong aversion to the cold would probably not feel restored in a snowy, mountainous environment. With these four components in mind, we can start to see why we may have gotten the results that we did. Viewing pictures of nature does not produce soft fascination, a sense of being away, or extent nearly as much as spending time immersed in the natural settings would. This could be why immersion in nature is so essential to producing the desired restorative results.

With that in mind it's also important to consider the limitations and possible future directions of our study. One limitation to our study that could be considered is the fact that we had participants view nature imagery for only 10 minutes, which is not comparable to the four days that participants spent out in nature during the LoTemplio et al. (2020) study. Further research could explore if spending a longer time viewing nature imagery would illicit different results. It is also possible that viewing a video of nature or that a more immersive virtual reality nature experience may influence amplitude of the ERN more so than static images do. These slightly more dynamic and immersive visual experiences may increase the amount of soft fascination and/or extent of the experience, thus potentially making them more restorative. On this note, it would be valuable to better understand the differences in nature immersion vs. viewing nature

imagery in the components of soft fascination, being away, and extent. Research could be done to measure these three components in those two conditions to see if there is in fact a significant difference in the components when you are spending time in nature.

Attention is becoming an increasingly important resource in our daily lives and so, finding ways to restore it effectively is essential. The more we understand how things such as nature affect us the better able we are to make choices that benefit us. Understanding that there is a difference between spending time in nature and viewing nature imagery changes the conversation around how important it is to protect natural spaces and how much we should invest in making them accessible to all people.

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# 69. Reflection on

# Undergraduate

## Reserach

# **Experience - Macey**

# Tilk

Macy Tilk (University of Utah)

Faculty Mentor: Mitchell Power (Geography, University of Utah)

I have really enjoyed my time as a UROP researcher. My mentor has a significant influence on how I approach research and I feel like I've learned a lot from him. I also feel as though this opportunity has helped me realize I would enjoy graduate school and research would be a good career path if I chose to do so. About the Author

Macy Tilk UNIVERSITY OF UTAH

# 70. Avalanche

# **Activity and Plant**

# **Biodiversity in the**

## Wasatch

# Mountains, Utah

Macy Tilk (University of Utah)

Faculty Mentor: Mitchell Power (Geography and Natural History Museum of Utah, University of Utah)

The Wasatch Mountains of Utah are widely known for their steep vertical relief and significant seasonal snowpack. These two factors create conditions that promote avalanche activity during winter storms. Physical factors such as temperature, snowpack depth, amount of relief and slope orientation are contributing factors for avalanche risk, however, what's happening below the snow, to plant communities, may be just as important. Limited research has been conducted on the impact to plant diversity and how seasonal avalanche cycles may influence biodiversity over space and time. This study explores several well-known avalanche chutes to see if avalanche frequency plays a role in biodiversity. To study this, vegetation communities were studied through line transects perpendicular to avalanche paths. Species inventories were collected along each transect and were categorized into six dominant vegetation types (herb, forb, grass, shrub, juvenile tree, adult tree) to provide an index of community diversity. Maps were then created to capture the frequency of avalanches in each research site. Surprisingly, data analysis of community plant diversity in three study sites suggest that more active avalanche chutes contain higher plant diversity while less active chutes recorded lower plant diversity.

#### Introduction

Avalanches have a large impact on both natural and human systems. It can be consequential for plants and animals in the path and can be dangerous for humans spending time in steep terrain. Avalanches have many positive attributes through creating habitat mosaics or early- and late-succession vegetation, providing shelter and forage for a wide range of species and ultimately increasing the diversity of plants and animals. Specifically, these habitats host numerous bird and small and large mammal species, including; squirrels, bears, moose, deer and elk. Therefore it is essential to understand how disturbances by avalanches improve or decay wildlife habitat and to disentangle which ecological processes are at work. In addition to biotic factors, abiotic components, including slope angle, changing winter snowpack and snow-water content are also critical conditions impacting these systems, especially as climate change increases the frequency of avalanches. Feedbacks within avalanche prone landscapes are an additional

aspect of this type of natural disturbance. For example, avalanches can shape ecosystems, including the composition of an ecosystem, which in turn, may have the ability to affect avalanche frequency, with large, old trees providing anchors and safety zones for organisms. For this undergraduate research project, I reconstructed plant diversity in avalanche through field-based chutes. line transect inventories of plant composition. Data gathered from two sites were compared to explore the impact of avalanche frequency on plant diversity. I hypothesize that biological diversity (an increased number of total tree and shrub species) is greatest in intermediate disturbed avalanche tracks and that avalanches are an agent of biodiversity.

#### Methods

Geographic information on avalanche frequency was used to generate study maps in Little Cottonwood Canyon. Individual avalanche chutes were broken into low, middle and high frequency for comparison on vegetation diversity. In order from least-to-most frequent avalanche activity, three sites were chosen, including; White Pine East (low), Little Superior (moderate), and Tanners Gulch (high). Data were collected by creating vegetation inventories along line transects, positioned perpendicular to avalanche paths.

Species including Symphoricarpos oreophilus, Populus tremuloides and Abies lasiocarpa were identified along the three study sites. These species were then categorized into six dominant vegetation types (herb, forb, grass, shrub, juvenile tree, adult tree) to provide an index of community diversity. Finally, data were summarized by compiling the biodiversity average score for each of the three avalanche chutes.

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Surprisingly, the Tanners Gulch chute had the highest biodiversity even though it has the highest frequency of avalanches. The mechanism behind this could be linked to avalanche frequency, vegetation history, geographic location, or elevational differences in the three study sites. Clearly, more work is needed to identify contributing factors on the linkages of avalanche disturbances on plant diversity in the Wasatch Mountains.




#### Biodiversity Averages in Avalanche Chutes

Avalanche Chute Name

#### Conclusion

This data has a wide range of applications including ecological and societal. This research was one of the first of its kind and additional work will shed light on the multiple factors influencing biodiversity in mountainous terrain. In this study, higher frequency chutes supported higher plant diversity, while the lower frequency paths had overall lower plant diversity. Plant inventory data at these fine spatial scales could help landscape managers make informed

decisions on the frequency and impacts of human-triggered avalanche control activities. Much research needs to be done in avalanche chutes to understand dominant mechanisms that influence plant and animal diversity over time, and this study provides a baseline for future analysis.

About the Author

Macy Tilk UNIVERSITY OF UTAH

## 71. Reflection on

## Undergraduate

### Research

## **Experience - Caleb**

## Woolston

Caleb Woolston (University of Utah)

Faculty Mentor: Anu Asnaani (Psychology, University of Utah)

These two years of working under Dr. Asnaani have truly been both eye-opening and rewarding. The past year of specifically researching safety behaviors has helped me better understand what the process of data collection and writing are truly like. Directly performing research has shown me what the real-world applications of science and how important implementing science into our education is. I have truly gained a greater understanding of what graduate school is like and bettered my resume to be a more well-rounded student. About the Author

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72. Impact of Trauma Exposure and Post-Traumatic **Stress Symptoms** on Baseline **Self-Reported Safety Behaviors** Versus **Observer-Rated Safety Behaviors During the Trauma Film Paradigm** Caleb Woolston

(University of Utah); Anu Asnaani (Psychology, University of Utah); Manny Gutierrez Chavez (University of Utah); and Kiran Kaur (University of Utah)

Faculty Mentor: Anu Asnaani (Psychology, University of Utah)

#### Abstract

Background: Post-traumatic stress disorder (PTSD) is a high burden disorder marked by safety behaviors (SB), which are covert or overt actions used to escape distressing feelings or places. However, literature suggests scores on observer-rated and self-reported SBs can be discrepant, creating a need to examine if this discrepancy exists in those who have had a traumatic exposure or in those with both trauma exposure and significant PTSD symptoms, to examine how SBs may present in PTSD and trauma. We expect that the presence of significant PTSD symptoms will be correlated with greater SBs and will result in a greater discrepancy between these two types of SB reporting standards. Methods: We used an analysis of variance (ANOVA) to examine difference scores between an observerrated measure of SBs performed by participants exposed to trauma-related videos and a self-reported measure of SBs at baseline. This was done across three groups: individuals with no trauma exposure (n=77), those with trauma exposure but minimal symptoms (n= 49) and those with trauma exposure and likely PTSD (n=24). Results: PTSD symptoms were correlated with self-reported SBs (r = .44, p<.001), but not observer-rated SBs (r = -.15, p=.30). The ANOVA revealed a significant difference between self-reported and observer-rated SBs for those with probable PTSD compared to the other groups, who did not show this discrepancy between types of SBs (F(2,101) =3.53, p= .03), such that those with likely PTSD had greater self-reported SBs than observer-rated SBs. Conclusions: We found that individuals with probable PTSD showed significant discrepancy in types of SBs, which suggests SBs may be more covert for individuals with PTSD and harder for observers to spot in clinical or research settings, highlighting that SBs may differ in those with PTSD and emphasizing the need to provide education on how individuals with PTSD may mask their avoidance.

**Keywords**: Posttraumatic stress disorder, safety behaviors, trauma, trauma avoidance, exposure therapy Impact of trauma exposure and posttraumatic stress symptoms on baseline self-reported safety behaviors versus observerrated safety behaviors during the trauma film paradigm

Posttraumatic stress disorder (PTSD) is a multi-layered and complex disorder. It is a pervasive and potentially debilitating mental illness defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association [APA], 2013) as the presence of each the following points: A) a direct or indirect exposure to a traumatic event, B) presence of intrusion symptoms associated with the traumatic event beginning after the traumatic event occurred, including disassociation episodes or reoccurring distressing memories and/or nightmares, C) avoidance of stimuli associated with the traumatic event, D) negative alterations in cognitions and mood associated with the traumatic event, E) marked alterations in arousal and reactivity associated with the traumatic event, including hypervigilance, and F) symptoms lasting over one month and not being due to effects of substances or medications (APA, 2013). One major factor that complicates the diagnostic process is that despite around 70% of the adult population experiencing a traumatic event at some point in their lives, such as military combat, sexual assault, or serious life- threatening illness or injury (National Institute of Mental Health, 2017), research has further shown that only around 8-15% of the general population will end up meeting diagnostic criteria for PTSD (Bryant-Genevier et al., 2021). This peculiar aspect of PTSD invokes a question as to what possibly differentiates patients who develop a PTSD diagnosis from those who do not following an exposure to trauma, a question that warrants scientific and empirical investigation.

Several current theoretical models of PTSD that exist to explain how PTSD occurs delineate the role that avoidance behaviors (i.e., the tendency to avoid thoughts, feelings or situations that remind one about the trauma) may play in maintaining symptom longevity of PTSD (LoSavio et al., 2017; Brewin & Holmes, 2003). Exposure-based treatments for PTSD target such behaviors, and involve guided, systematic, and repeated confrontation with feared stimuli, with significant evidence of their efficacy for PTSD (Brown et al., 2019). One particular target in exposure therapy is eliminating a type of avoidance known as safety behaviors (SBs, i.e., overt or covert actions performed to prevent, escape, or minimize a feared catastrophe and/or associated distress; VandenBos, 2015). Essentially, individuals utilize SBs to cope with the anxiety and distress of unpleasant feelings or the risk of feared outcomes associated with traumatic feelings, places, or events (Helbig-Lang & Petermann, 2010).

Those who use SBs intend to use them as a short-term means to relieve anxious feelings and symptoms (Helbig-Lang & Petermann, 2010). However, emerging research has shown that utilization of SBs has been shown to maintain those symptoms, rather than the intended purpose of reducing anxiety and fear (Allen et al., 2020). This leads to persistence of such symptoms, showing that the short-term relief provided by SB usage does not necessarily translate to long- term relief of mental health symptoms (Blakey & Abramowitz, 2016; Forsyth et al., 2016). Since SBs are a hallmark of trauma disorders (Blakey et al., 2021), the study of SBs is highly relevant to understanding the multi-layered relationship between PTSD and trauma exposure. The frequency of patient usage of SB has been shown to be a significant predictor of a PTSD diagnosis as well a correlate of severity of symptoms (Karamustafalioglu et al., 2006; Freeman et al., 2013; Goodson & Haeffel, 2018). Additional research has shown an association between SB engagement and traumatic intrusions in particular (McClure et al., 2019).

Despite these empirical findings, patients with PTSD and/or trauma exposure who utilize SBs are often hesitant to eliminate them. One likely explanation for this is that patients may misattribute positive outcomes in anxiety- and traumaprovoking situations to the use of SBs. For example, an individual who becomes anxious in a public place may exhibit the SBs of bringing anti-anxiety medication in anticipation of feeling anxious in public. If the anxious feelings do not arise, the patient may misattribute this to bringing the medications as opposed to other factors. In essence, the patient is assigning causality to the utilization of the SBs as the reason for maintaining safety rather than an actual cause of the traumatic or anxiety-inducing event not occurring. Furthermore, patients utilizing SBs seldom understand their actual role in maintaining symptoms rather than alleviating symptoms (Helbig-Lang & Petermann, 2010).

Safety behavior measuring methods can be divided into two distinct categories: subjective, or patient self-report methods, and objective observer-rated methods. Self-report methods are usually offered by clinicians and researchers as a questionnaire wherein the participant voluntarily self-reports frequency of SBs, usually during day-to-day activities. Objectively reported methods are those that are coded by an observer - for example, based on SBs performed during a task or video clip designed to elicit an anxious or trauma-like response (Olatunji & Fan, 2015). A more recently developed method of objectively measuring safety and avoidance behaviors is rating behaviors made by participants in a computer simulation game involving avoidance-like decision making in simulated events or situations (Myers et al., 2016). While methods vary, the goal of observation methods is to measure frequency of SBs' usage and these objective measures are most often used to measure correlations with mental health symptoms.

Despite numerous empirically supported methods of measuring SBs, the process of examining the role trauma exposure and PTSD symptoms have on SBs is significantly complicated by research showing that there exists a significant discrepancy between subjective participant reporting of SBs and observer ratings of such behaviors in fear-based disorders more broadly. Specifically, Rowa et al. (2015) found that selfreported SB scores were greater than observed SBs when rated by a coder during a recorded speech test. Research done by Blakey et al. (2020) in the United States has shown that this discrepancy on reporting SBs can vary by region with cultural attitudes significantly influencing how some self-report SBs present. To our knowledge, however, there has been limited work that experimentally examines whether and how this discrepancy exists specifically in the context of PTSD when compared to those with a no traumatic exposure and/or a traumatic exposure that does not manifest as PTSD.

While SBs were only initially examined in the context of the broader class of anxiety disorders (i.e., generalized anxiety disorder and obsessive-compulsive disorder), there is growing interest in examining the role they may play in maintaining PTSD symptoms and diagnosis. However, there exists a dearth of research examining if this discrepancy also exists in participants with simply a history of an exposure to trauma or what role, if any, PTSD symptoms may play in explaining this reporting discrepancy. A major focus of exposure therapy targeting and eliminating symptom-maintaining is SBs, necessitating further examination into whether trauma exposure and/or PTSD symptoms may be associated with discrepancies between patient- reported as opposed to clinician-rated methods of safety behavior utilization.

Given this gap in the literature, the present study sought to examine if a discrepancy between selfreported and/or objectively measured, observerrated SBs exists, and if this may be linked to an exposure to trauma, or the presence of significant PTSD symptoms. There were three aims of this present study: the first aim was to measure if the presence of PTSD symptoms were correlated with self-reported and/or observer-rated SBs. We hypothesized that there would be a significant correlation between the variables of both observerrated and self-reported safety behavior in

individuals reporting greater PTSD symptoms. The second aim of this study was then to examine if there were any significant differences in the selfreported and observer-rated SBs across three groups: those with no trauma exposure, those with a past trauma exposure but few PTSD symptoms, and those with trauma exposure and significant PTSD symptoms. Based on previous literature examining the role SBs play in symptom severity and maintenance, we expected that the groups with trauma exposure and minimal PTSD symptoms and probable PTSD would have a significantly greater number of both kinds of SBs compared to those with no trauma exposure. The third and final aim herein was to test if a discrepancy between the two types of safety behavior reporting methods would be associated with specific group membership. We lastly hypothesized that a difference in reporting discrepancy between types of SBs would be statistically associated with having probable PTSD only, in line with research in other fear-based disorders (Blakey et al., 2020).

#### Methods

Participants

Institutional Review Board approval was given for all research and procedures performed in this study. Signed informed consent was received from all participants and all data were deidentified. Participants (N=150) for this experiment were recruited as part of a larger study conducted by a research laboratory at a public university in the Mountain West region of the United States. Recruitment for this study was done via SONA, a centralized online recruitment program for recruitment of undergraduate students, for which participation in this study was awarded course credit. The sample included participants identifying ethnically as 62.0% white,19.7% Hispanic or Latino, 8.3% Asian/Pacific Islander, 1% Native American, 2.2% Black, and 7.3% other races. Demographics additionally included 24.2% first-generation college students and 34.6% upper income-class participants.

Materials

Trauma Film Paradigm. This task consisted of one of two different video clips viewed by study participants. The videos were both five-and-a-half minute long videos of potentially fatal car crashes. Specifically, one of the videos was a compilation of various scenes of car crashes with injuries explicitly shown and the other was of unedited footage of one particular car crash shown in a full five-and-a-half-minute long interval. These clips have been shown to elicit anxious feelings and were designed to evoke SBs in participants who utilize them (Olatunji & Fan, 2015). Due to a desire to maintain social distancing in light of the ongoing COVID-19 pandemic, these videos were displayed on the participant's own computer while on a live video Zoom call with a lab assistant.

Self-Reported Safety Behavior Assessment Form (SBAF). The Safety Behavior Assessment Form (SBAF) was utilized in this study to measure subjective self-reported safety behavior utilization. The SBAF is a self-report questionnaire wherein participants respond to a 41-item battery regarding various SBs they may potentially utilize in day-today activities, including questions/statements such as, "Do you over-plan for everyday events?" and "I stay on the outside of crowds and/or monitor for exits or escape routes". Participants rate each question on a scale of zero, one, two, or three, with corresponding to "never", number each "sometimes", "often", or "always", respectively (Goodson et al., 2016). Each question is further categorized into one of four subscales, in addition to a total overall score. These subscales are vigilance (7 items), social (7 items), health (8 items), or PTSD (12 items) and seven unaffiliated questions contributing to the final score, and the range of total scores was 0 to 123. Previous research has shown that this measure has high levels of internal consistency and test-retest reliability (r = .76), as well as an excellent level of internal consistency  $(\alpha = .92)$  and may be used by clinicians and experimenters to measure patient SBs for treatment/research (Goodson et al., 2016). Internal consistency of the SBAF total measure in the current sample was excellent ( $\alpha$ =90).

Objectively-Rated Safety Behavior Coding. The

observer-rated SB measure was scored via a behavioral coding measure specifically designed for this study. Specifically, videos of study participants engaging in the trauma film paradigm were recorded and later coded by undergraduate research assistants trained to code for observed SBs by a licensed clinician. Research assistants were specifically trained to record the frequency and type of various observed participant SBs, including items such as how often the participant looked away from the screen or how often they engaged with the lab assistant to feel safer. Training procedures included having coders rate three test subjects with 90% accuracy to the scores obtained by the training clinician, and only being cleared to conduct study coding upon reaching this threshold with three consecutive test participants. There were ten questions rated by the coders on a scale of zero, meaning "not at all", to four, meaning "very often", on how often these behaviors were exhibited over the viewing of each distressing film. These included the observer rating behaviors such as delaying starting the films and intentionally engaging the observer to avoid the film. Additionally, some questions included a scale measuring intensity of the behaviors, also measured on a zero, meaning "never" to four, meaning "very intense behavior". The total score had a range of scores of zero to forty. Data regarding observed SBs were recorded in the survey software program Qualtrics to generate a

total score of observable SBs during each distressing film task.

PC-PTSD-5. The PC-PTSD-5 is a five-question clinical assessment self-report questionnaire that assesses for probable PTSD. The questionnaire asks participants questions on a yes or no scale, initially asking if the participant has experienced any one event of a list of traumatic events such as combat. having been in an accident, having a close loved one die through homicide or suicide, among others. If the participant answers "yes", they are then asked five follow-up questions that ask details about the presence of PTSD symptoms in the past month (yes or no). This portion of the questionnaire includes questions such as "had nightmares about the event(s) or thought about the event(s) when you did not want to?" and "have you been constantly on guard, watchful, or easily startled?", to which participants answer "yes" or "no". Scores can range from 0 to 5, with those scoring three or higher categorized as having "probable PTSD". Previous research with the PC-PTSD-5 has shown good testretest reliability (r = 0.83) as well as strong internal validity (r=.083; Prins et al., 2016). Internal consistency of this measure in the current sample was found to be good ( $\alpha$ =.79).

Procedure

After informed consent was received from participants, individuals were asked to fill out the SBAF and PC-PTSD-5. Participants were then given one of the two distressing films from the trauma film paradigm to view, which they were asked to complete either before or after a separate breath holding task (not examined in this study), in addition to subsequently completing one of three interventions (interoceptive exposure, mindfulness, and a positive self-efficacy induction) occurring after the trauma film paradigm that were aimed at improving participants' distress tolerance (which were also not the focus of the current paper). They then were given the second trauma video to view either before or after another breath-holding task (with counterbalanced order; this second video was also not examined in the current study). During these tasks, participants' behaviors were monitored and recorded via the Zoom meeting. After completion of the entire study, all participants were debriefed and provided with support resources if they requested, as this study included exposure to distressing videos and risked being emotionally upsetting to participants. The recorded videos of participants viewing the trauma film videos before and after intervention were then later coded for SBs by the aforementioned coders. As mentioned above, for the current study, only SBs observer-coded for the trauma film viewed before the provided intervention were examined and compared to participant self- reported SBs at baseline.

Data Analysis

All data were analyzed via the statistical software

program SPSS Statistics version 27. Initially, we ran the suggested scoring guidelines to organize participant data into three groups of either nontrauma exposed, trauma-exposed with minimal PTSD, and trauma exposed with probable PTSD, based on scores on the PC-PTSD-5 to address aims 2 and 3. Specifically, participants who answered "no" to the first question, i.e., if they had a previous exposure to a traumatic event, were placed in the non-trauma exposed group (n = 77). Those endorsing a trauma exposure but answering 'yes' to only one or two of the remaining questions were placed into the trauma-exposed group (n = 49). Participants reporting a trauma exposure and endorsing three or more PTSD symptoms on the PC-PTSD-5 were placed into the probable PTSD group (n= 24), based on previously suggested scoring guidelines for this measure (Williamson et al., 2022).

Data regarding subjective self-reported SBs as measured by the SBAF (including the vigilance, social, health, and PTSD subscales, and the total score) and the observer-coded safety behavior usage data were correlated with the score on the PC-PTSD-5 (Aim 1). Following this, an analysis of variance (ANOVA) was conducted to test if there were significant differences in self-reported or observer-rated SBs across any of the three trauma exposure groups (Aim 2). Finally, to examine whether there was a significant difference between the discrepancies on both types of SBs and whether this was associated with any of the trauma groups (Aim 3), the self- report and observer-rated measures were compared by first z-scoring the SBAF total scores and the video-coded observer scores and calculating the difference between the two sets of z scores. This difference z score was then compared across the three groups of participants via a between- groups ANOVA, followed up with pairwise comparisons to examine any significant differences between groups in the discrepancy between self-reported and observer-rated SBs, at the significance level of p=.05.

Results

For Aim 1, correlations regarding SBs for both the self-report and observer-rated measures with the PC-PTSD-5 score can be found in Table 1. Briefly, observer-rated scores were not found to be statistically associated with PC-PTSD-5 scores (see Table 1). However, each of the subscores on the self-reported SBAF, other than the PTSD subscore and the total score were found to be positively correlated with greater scores on the PC-PTSD-5.

| Table 1. PC-PTSD-5               | symptom | s correlati | on with s | elf-reporte | ed safe | ety behav | viors |       |       |   |
|----------------------------------|---------|-------------|-----------|-------------|---------|-----------|-------|-------|-------|---|
|                                  | n       | М           | SD        | 1           | 2       | 3         | 4     | 5     | 6     | 7 |
| 1.PC-PTSD Score                  | 74      | 1.85        | 1.76      | _           |         |           |       |       |       |   |
| 2. Observer-rated                | 129     | 2.11        | 1.84      | 15          |         |           |       |       |       |   |
| score                            |         |             |           | 15          | _       |           |       |       |       |   |
| <ol><li>SBAF Vigilance</li></ol> | 137     | 8.15        | 3.89      | .26*        | .13     | _         |       |       |       |   |
| 4. SBAF Health                   | 137     | 4.47        | 3.33      | .38**       | .10     | .38**     |       |       |       |   |
| <ol><li>SBAF Social</li></ol>    | 137     | 8.84        | 4.20      | .38**       | .02     | .44**     | .30** | _     |       |   |
| 6. SBAF PTSD                     | 137     | 13.62       | 5.76      | .01         | .01     | .91**     | .40** | .73** | _     |   |
| 7. SBAF Total                    | 137     | 21.46       | 8.76      | .44**       | .05     | .80**     | .70** | .79** | .90** | — |
|                                  |         |             |           |             |         |           |       |       |       |   |

Note: N =178. M and SD signify mean and standard deviation, respectively. \*p < .05, \*\*p < .001. SBAF 41= Safety behavior assessment form-41.

Table 1. PC-PTSD-5 symptoms correlation with self-reportedsafety behaviors

Table 2 presents mean scores of observer-rated and selfreported SBs across the three trauma groups for Aim 2. Briefly, the ANOVA revealed no significant differences among the three groups on observer-rated SBs, F(2,128)=.25, p=.78. Regarding SBAF scores, A significant difference across groups was found in the SBAF scores of health subscore (F(2,136)=6.13, p<.01) and total score (F(2,136)=5.39, p<.01) only, such that the probable PTSD group showed increased mean scores on the health subscale and the SBAF total score (see Table 2). The vigilance subscore, F(1,136)=.1.57, p=.21, the social subscore, F(2,136)=2.85, p=.06, and the PTSD subscore F(2,136)=2.56, p=.08, were all found to not be significant across the groups.

|   | St<br>No trauma<br>exposure group<br>n=77 |      | tudent Participant Gro<br>Trauma exposure<br>& no PTSD<br>n=35 |      | oup<br>Probable PTSD |       | F(2,134) | $\eta^2$ |
|---|---|------|--|------|----------------------|-------|----------|----------|
|   |   |      |  |      | <i>n</i> =24         |       |          |          |
|   | М   | SD   | М  | SD   | М                    | SD    | _        |          |
| SBAF Vigilance                                | 7.86                                      | 3.93 | 7.90   | 3.98 | 9.42                 | 3.50  | 1.57     | .02      |
| SBAF Health                                   | 3.89                                      | 2.78 | 4.22   | 3.13 | 6.54                 | 4.30  | 6.13*    | .84      |
| SBAF Social                                   | 8.43                                      | 3.69 | 8.48   | 4.39 | 10.67                | 4.72  | 2.85     | .04      |
| SBAF PTSD                                     | 13.02                                     | 5.71 | 13.24  | 5.80 | 16.00                | 5.45  | 2.55     | .04      |
| SBAF Total                                    | 20.17                                     | 7.68 | 20.60  | 8.69 | 26.63                | 10.01 | 5.39*    | .08      |
| Observer-rated<br>SBs during film<br>paradigm | 2.06                                      | 1.83 | 2.29   | 1.90 | 1.94                 | 1.84  | .25      | .004     |

 Table 2. Means of safety behaviors across trauma groups

*Note:* \*p < .05, *M* and *SD* signify mean and standard deviation, respectively.

## Table 2. Means of safety behaviors across trauma groupsStudent Participant Group

For Aim 3, to analyze the difference in the discrepancy between observer-rated and self- reported SBs, the between-groups ANOVA of the z-scored data revealed a significant difference in

means between the two types of SB reporting in the probable PTSD group only (difference of means = .82), such that participants with probable PTSD had greater SB scores on the SBAF than their observer ratings of their SBs during exposure to the trauma film paradigm. Pairwise comparisons further confirmed that this discrepancy was statistically greater for the probable PTSD group when compared to the no trauma exposure group (F(1,65)=7.18, p<.01), and the trauma exposure with no PTSD group, (F(1,51)=5.23, p<.05). This difference in the discrepancy between self-reported versus observer-rated SBs was not found between the no trauma exposure group (difference of means= -.15) or the trauma exposed but minimal symptom group (difference of means=.20). The non-trauma exposed and the trauma exposed with minimal PTSD symptom group did not differ statistically from one another (difference of means = -.13, F(1,82)=.03, p=.87).

#### Discussion

An increased presence of PTSD symptoms was not correlated with higher observer-rated SBs; however, it was correlated with higher scores on all subscales except PTSD on the self- reported SBAF (i.e., self-reported SBs). This is partially consistent with literature explaining the role SBs play in symptom severity and maintenance. The hypothesis of associations of differences in the SB reporting methods across the groups was also partially supported, whereby significant differences were found across the groups for SBAF health subscale and the total scores, such that there were higher selfreported SBAF scores in these domains for those with probable PTSD only. The difference between SB reporting scores was not significant for observer-rated or the SBAF vigilance, social, or PTSD subscore SBs, and those with trauma exposure with minimal PTSD symptoms did not show significant elevations on any of the SB types compared to those with no trauma exposure. The final hypothesis was fully supported by the data, where a significant discrepancy was found between selfreported and observer-rated SBs, such that only those with probable PTSD higher scores obtained via the self-reported methods compared to observer-rated methods of SBs. This pattern was not found in either groups of those with no trauma exposure or those with trauma exposure but minimal PTSD symptoms by the pairwise comparisons.

Evolutionary theory may assist in explaining why patients with likely PTSD may utilize SBs that are more covert, rather than overt: specifically, background literature postulates that PTSD is one of the most stigmatized disorders in the DSM-5 (Krzemieniecki & Gabriel, 2019). This high level of stigmatization may give patients with PTSD greater reason to hide any behaviors (including SBs) that may clue others into whether the individual has PTSD. In short, patients with PTSD may have learned to adapt by utilizing covert SBs (rather than overt and thus more easily observable behaviors), possibly in an effort to preserve the guise of public functioning. Findings in this study further underscore the importance of examining how the elimination of all safety behaviors (overt and covert) may improve outcomes in exposure therapy for PTSD.

Further, the misattributed advantages of utilizing covert SBs a create a need for education regarding the unique presentation of SBs used by those with PTSD. These findings may show that people with

PTSD are harder to identify in public or that the avoidance conferred by their symptoms may not be readily apparent to those in their social and community networks. Educating the public on the fact that just because an individual may not, on the surface, seem to have PTSD symptoms or engage in overt trauma avoidance, that such a lack of overt SBs does not necessarily translate into an individual not having any PTSD symptoms. This finding emphasizes the need for empathy from the public as well as general societal education on the need to be aware that those with PTSD may still benefit from therapies that help these individuals approach trauma-related stimuli. An additional implication of this research is that PTSD SBs may present in a unique manner. Our current methods of treating PTSD could likely benefit from these findings which highlight the unique ways PTSD presents itself. This finding underscores the need to further educate clinicians and researchers (beyond the general public) on the nature of PTSD SBs and how they present differently from those without PTSD. Increasing our understanding of SBs may effective in also assist more assessment. examination, and treatment of PTSD.

In addition to clinical implications, these findings are consistent with previous literature that postulates that a traumatic exposure is a very different phenomenon than PTSD in many significant ways (Zohar et al., 2011; Lewis et al., 2019), as was clear from our findings that having exposure alone (in the absence trauma of significant PTSD symptoms) did not show higher SBs. That is, PTSD is differentiated from traumatic exposure (specifically without PTSD) by its persistence and debilitating symptomology as well as a disturbance in one's functioning (Ehring et al., 2008, Miao et al., 2018). In addition, trauma exposure measures in research and clinical settings typically seek to measure the presence of an exposure to a traumatic event, and do not serve as a diagnostic tool whereas a PTSD measurement seeks to determine a matching symptom presentation as determined by the DSM-5, generally for diagnostic purposes (Hall & Hall, 2007). While SB usage may play a role in traumatic exposure evolving into PTSD, further research is needed to examine if having a difference in the types of SBs used plays a role in the relationship between exposure to trauma and subsequent PTSD diagnosis.

Strengths of the present study include good sample sizes considering population size of those with PTSD, good internal consistency of measures, use of well-supported measures, and overall good generalizability of the sample. Yet, while this study such notable strengths, there are some has limitations that ought to be addressed in future studies. Specifically, the current sample was limited only college students and had to an overrepresentation of higher income students,

which somewhat limits the degree to which we can generalize these findings to middle or lower income as well as non-college educated populations, communities and age groups. Also, the nature of examining SBs outside of everyday life may skew how observer-rated SBs present. Specifically, it could be that we were measuring different contexts for SBs with the SBAF examining SBs performed in daily activities, while the observer-rated measure was specifically in the context of a stressful film task. Additionally, the trauma paradigm used in this study was a compilation of car accidents, and while most would find these videos upsetting, different videos could elicit different responses based on one's unique trauma (e.g., those with a sexual assault traumatic exposure would not have the same traumatic reaction as those with a trauma from car accidents). However, due to background literature suggesting the trauma film paradigm does indeed elicit traumatic reactions (Olatunji & Fan, 2015), we believe the effect of incongruent traumatic reactions with the car accident films is very limited.

Future studies should examine the specific factors that may explain why some SBs, but not all, are utilized by those with PTSD and whether we can replicate the current findings that some scores do not have a significant difference in the reporting discrepancy while others do (e.g., why self-reported vigilance was not found here to be significantly

correlated with PTSD and other types were). Additionally, further research examining what other variables intersect with SBs and that may differentiate those who do develop PTSD from traumatic exposure and those who do not is needed (Dunmore et al., 2001; Ehring et al., 2008). Future studies should also focus on replication, such as utilizing samples that include the general population rather than solely college students and better sample representation of ethnic minorities to more closely examine possible cultural or generational/age-related differences the in presentation of various types of SBs. Despite these limitations and the need for additional research, this study enhanced understanding of how SBs present in college students with probable PTSD and how they differ from those without PTSD. Clinicians can benefit from understanding the diverse nature of SBs and how to recognize the diverse ways they manifest in those with probable PTSD.

#### Acknowledgements

I would like to thank the Treatment Mechanisms, Community Empowerment, and Technology Innovations (TCT) Lab at the University of Utah (PI: Asnaani) for the opportunity to conduct this research. I want to especially thank Ifrah Majeed, the research coordinator, for overseeing data collection, entry, and remote implementation of participant recruitment. I also want to thank my fellow undergraduate research assistants Ally Askew, Jiayu Gao, Angela Pham, Sami Soufi, Tracey Tacana, Will Tanguy, and Michael Wasser for assisting in coding and data collection. I want to additionally thank my graduate advisors Kiran Kaur, Jennifer Isenhour, and Manny Gutierrez Chavez for their help in the research and editing process. I am also very thankful to Dr. Anu Asnaani for the opportunity to examine this study idea, and for accommodating an Honor's Thesis despite the many challenges with the ongoing COVID-19 pandemic. Similarly, I am thankful to Dr. Lisa Aspinwall and Dr. Trafton Drew who serve as the department's Honors Thesis Advisors and provided invaluable feedback on the final paper. Last but not least, I would like to thank all of the research participants for assisting in this research by participating in our somewhat challenging research procedures.

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## 73. Reflection on

## Undergraduate

## Research

## **Experience - Shane**

## Denherder

Shane Denherder (University of Utah)

Faculty Mentor: Elisabeth Conradt (Psychology, University of Utah)

This experience has introduced me to the process of forming research questions, collaborating with mentors, forming hypotheses, running statistical analyses, and preparing and submitting a manuscript. Importantly, this has prepared me for graduate studies in clinical psychology. About the Author

Shane Denherder UNIVERSITY OF UTAH

**DEPARTMENT XIII** 

# College of Social Work
74. Scoping Review

of Anti-Racism

**Programs and** 

**Practices Within** 

**Higher Education:** 

Institutional

Accountability and

the Promises of

Equity, Diversity,

## and Inclusion

Audrey Pozernick (University of Utah) Faculty Mentor: Tiffany Baffour (Social Work, University of Utah)

The concept of anti-racism training in higher education is relatively new in the United States and Canada. Despite the term anti-racism appearing as early as 1970, anti-racism training focused on systemic factors to dismantle racism and oppression did not emerge until the early 2000s. Higher education institutions have gradually incorporated anti-racism training in response to larger social movements (Williams & Wade-Golden, 2009). However, Black, Indigenous and People of Color (BIPOC) student and faculty continue to experience significant barriers to well-being due to systemic racism. First, hate violence and racial trauma caused by these experiences negatively impact BIPOC students and faculty (Irwin et al., 2022). Secondly, the needs of today's BIPOC students and faculty, who are more likely than their white peers to experience poverty, housing, and food security, and who often experience inequalities in work demands, are not met (Diamond & Stebleton, 2017; Cukier et al., 2021). Additionally, there has been little progress in the development of anti-racist practices that facilitate improved organizational outcomes. These factors demonstrate a critical need for evidenceinformed organizational strategies that higher education systems can utilize to develop competencies to accelerate racial equity to improve institutional outcomes. This study looks at data from American and Canadian universities (N=166) within the National Association of Diversity Officers in Higher Education (NADOHE) to determine a count of annual reports (2019-2020 or 2021-2022) and if there was a research methodology utilized.

Key words: Equity, Diversity and Inclusion, Anti-Racism

Organizational Strategies and Accountability, Evidence-based Anti-Racism Practice, Racial Justice, Higher Education.

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