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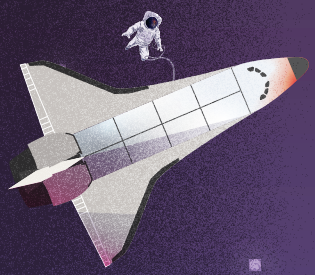

If we knew what it was we were doing, it would not be called research, would it?"

– Albert Einstein

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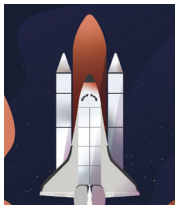

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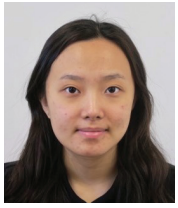

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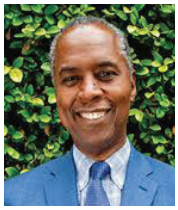

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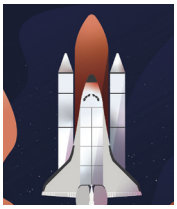

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EDITOR'S **LETTER**

Dear Reader,

Thank you for being a part of the fourth issue of the Challenger Research Journal. To provide you with a brief introduction to Challenger's history, the Challenger Research Journal was established in 2019 by UC San Diego undergraduate students, under the guidance of the Undergraduate Research Hub. Since then, four volumes have been published, including this one. Our foundation rests on a mission of inclusivity and advocacy for the advancement of student researchers, especially those who are minorities, underserved, and underrepresented in education and in research specifically. We take pride in publishing the works of Challenger scholars on E-Scholarship, along with other platforms, where their recognition and research are highlighted.

The collection of papers we received and accepted this year profoundly delves into and explores some of the most pressing issues in society and our rapidly evolving digital world. Starting with our current concerns regarding the battle against misinformation and the selective portrayal of information to the advantage or detriment of certain parties, student author Zerui Pan discusses the impact of state censorship in China and Japan during the 1930s and 1940s on culture and traditions. Pan underscores the adverse effects of censorship as it resulted in the suppression and attempted eradication of certain film subgenres in the respective countries mentioned. While this paper focuses on a specific segment of society subjected to censorship, it emphasizes the significance of freely expressing ideas without selective or complete censorship.

Furthermore, in light of the recent widespread introduction and accessibility of artificial intelligence (AI), student author Christian Flores explores the public perception of artificial intelligence in high-risk domains. As AI becomes increasingly integrated into various aspects of society, this paper significantly contributes to our comprehension of the discourse surrounding AI and the associated issues. Flores identifies areas for improvement in establishing more ethical AI systems.

Continuing with the scholarship and research into the utilization of gene editing techniques as a means of providing medicines and understanding diseases, student author Mohnish Alishala aims to understand the mechanism of a surface receptor present during tissue injury in macrophages through Cas9 gene editing. Gene editing has become a promising tool that alters our current scientific understanding, and Alishala's paper demonstrates an example of the myriad uses of gene editing.

This volume represents the culmination of a year's worth of tireless work by our board members, student authors, mentors, and advisors. Together, we are immensely proud to present this volume and all the individuals who have contributed to bringing it to life. We sincerely thank you for your support in reading and engaging with the papers authored by our talented students.

Sincerely,
Farah Haleem and Sally Guan
Co-Editors-in-Chief

In Commemoration of **Dr. Ronald E. McNair**

Ronald McNair was not born under particularly unusual circumstances. He grew up in Lake City, South Carolina during the 1950's when segregation dominated the lives of African Americans in the South, forcing most to live in poverty and with limited access to resources. Despite his humble beginnings, McNair would go on to earn a PhD in Physics at M.I.T., become one of the first African American astronauts, and be honored posthumously by the US Congress with a federal education program dedicated in his name.

From a young age, Ronald McNair demonstrated an unshakable will to pursue his dreams in the face of adversity. Growing up in South Carolina during the 1950s, McNair, like other blacks at the time, was prohibited from using the same facilities as whites. Despite this, one day when McNair was nine years old, he went to the local public library to check out books on advanced science and calculus. As he stepped into line, the librarian refused to let him check out the books, instead demanding he leave. A young, passionate McNair would not budge, and police were eventually called along with his mother. In the end, the policemen allowed McNair to check out his books, and the library has since been renamed after him, in honor of the boy who refused to yield. McNair refused to let his social surroundings dictate his future endeavors.

While growing up, McNair's interest in space exploration would manifest with the launch of Sputnik in 1957, and later grow with Star Trek, which featured a diverse cast. Excelling in his studies at school, he became the first in his family to attend college at North Carolina A&T and would later matriculate at M.I.T., where he earned a PhD in Laser Physics. McNair would go on to be selected by NASA to become a crew member of the Space Shuttle Challenger, as well as the second African American to reach space. While in space, he served as a mission specialist and operated the robotic arm of Challenger. Unfortunately, McNair was one of seven crew members who were killed in January of 1986, when Challenger exploded moments after lift-off due to a malfunction in the rocket's boosters. McNair's legacy endures through the education initiatives founded in his name, and his life serves as an inspiration for individuals who are born into disadvantaged and similarly challenging circumstances.

"Before you can make a dream come true, you must first have one."

—Dr. Ronald E. McNair

STUDENT **PAPER** 1

Building Trust in the AI Ecosystem by Re-Evaluating Public Perception

Researcher: Christian Flores | Mentor: Dr. Sean Kross

ABSTRACT

Artificial intelligence systems leverage large datasets with iterative processing algorithms that identify patterns to create an additional layer of expertise. This transformational power operates in tandem with ethical risks. The dominant narrative behind AI is simultaneously stigmatized and misunderstood: with exponential growth of the ubiquitous technology leaving public awareness in the dust, it's becoming increasingly important to balance enthusiasm for AI's enormous promise with a sober understanding of its moral risks. This study seeks to characterize the public opinion of AI in high-risk, domain-specific applications. To that end, a poll was administered to American adults. The results of the study reveal that the great majority of survey respondents have a neutral or optimistic perspective on AI in particular high-risk domains. The study concludes by presenting a standard heuristic for understanding public perception where ethics may fail to preserve a human factors' approach. In this way, researchers and developers can undertake coordinated efforts to mitigate the harm caused by AI while promoting rational optimism in vulnerable populations.

INTRODUCTION

AI systems leverage machine learning algorithms to maximize the potential of big data. However, deploying machine-learning algorithms at scale comes with risk. This, in part, can be explained by the inability for artificial intelligence to replicate human capabilities including individual flexibility, context-relevant judgements, empathy, as well as complex moral judgements (Webb et al., 2021). Now more than ever, the dilemma of socially unaware AI is synonymous with scandal (Dressel & Farid, 2018) (Bartlett et al., 2022). With eager news sources and online publications acting as doomsayers, the narrative surrounding AI has shifted from harnessing life-changing potential to impending doom. A story of promise and peril. The pressure is mounting to design ethical AI: a report made by researchers at Cambridge and Oxford enumerates a number of priority research areas for AI development, one of them highlighting the importance behind balancing optimism about the vast potential of AI technology with a level-headed recognition of the risks involved (Brundage et al., 2018). Prior research has examined how the general public perceives the impact of the technology (Müller & Bostrom, 2016) (Grace et al., 2018). However, the research lacks a standard method for understanding public perception and sentiment behind AI. Furthermore, prior research has not concentrated on AI application in specific domains. Instead, the vast majority have opted to monitor perception and sentiment on the topic of AI in a broad sense, using select media news source coverage and broadly encompassing survey topics.

In the study presented in this paper, 280 adults aged 18 or older in the United States completed an online survey. To promote generalizability, the survey sample was demographically balanced to include a wide range of respondents with different gender, age, and level of education. The research inherently promotes a risk-benefit evaluation strategy to evaluate the data critically. We investigate one essential research question:

RQ) What are the attitudes and sentiments towards AI-application in high-risk domains?

The results from the poll indicate that the great majority of respondents had a neutral or optimistic perspective on AI in particular high-risk domains. In addition, by analyzing the attitude of survey respondents, we may conduct a more thorough analysis of perception. While there is a greater range of keywords used to describe "negative" attitudes, "positive" words were more prominent in the data, as determined by sentiment analysis. The findings of this study shed light on how the general public perceives high-risk domains in the AI ecosystem. The results also give developers and lawmakers who regulate AI with a compass for future development and governance.

In general, survey respondents accept both the positive and negative aspects of domain-specific AI applications, as opposed to accepting only one side. However, further investigation reveals that emotional evaluation questions across all domains tend to have a positive sentiment. This outlook reveals wide-ranging insights with an overall optimistic tone on the future of AI.

A risk-benefit analysis is a robust tool for determining public acceptance and support for a particular technology. The experimental heuristic described in the Discussion section could assist researchers in detecting whether AI could aid researchers in determining if AI applications are losing public favor.

Related Literature

Recent research tries to elucidate the underlying effects of algorithm-driven AI across many aspects of modern life. A growth in large survey studies attempts to address the disconnect between the disruptive technology and the general population. Despite a gradual shift from “robot apocalypse” and “automation boon” tropes to a more open-minded approach in discourse surrounding the subject, the societal effects of AI continue to draw intense public attention (Littman et al., 2021).

Analysis on Mass-Media Discourse

In the past, text mining techniques have been used to determine how the media feels about AI. Fast and Horvitz conducted a long-term study of how the public felt about articles published by the New York Times between January 1986 and May 2016, which added up to a staggering 3 million pieces. The purpose of the study was to determine how people's hopes and fears regarding AI have evolved over time. Indicators for a set of hopes and fears about AI are collected in the study. To accomplish this, they examined how ideas have changed over time by searching article text for positive or negative keywords. This enabled them to sort article topics into two primary groups: those that express a hope or a fear. The research article concludes that existential fear and worry about a number of AI applications are on the rise. In addition, there has been an increase in ethical concerns regarding AI over the past three decades, which have been driven in part by existential concerns. The essay concludes with a summary report on the optimistic or pessimistic perceptions of AI-related publications. Surprisingly, the study reveals a cheerful outlook for the future of AI. Since 2009, there has been a sharp increase in the number of public discussions about AI, with the majority of media sources expressing optimism (Fast & Horvitz, 2017).

Other studies of mass media discourse have tracked the evolution of AI as covered by major news media outlets. Zhai et al. analyze five major news media outlets over the past three decades using seven dimensions: scientific subject, keyword, country, institution, people, topic and opinion polarity. While the study acknowledges that AI has become an important force in the new era, the public's perception of AI has been contested. According to a widely held belief in the media, AI is the driving force behind the modernization of conventional industries. However, the notion of AI as a "humanized" technology is not yet widely accepted. This can be summed up by one single insight: when we transfer the right of judgment to computer systems, there will be a number of moral and ethical dilemmas involved (Zhai et al., 2020).

Chuan et al. explores framing theory in journalism and science communication on the topic of artificial intelligence. The objectives of the study were to evaluate how the topic of AI was presented in major American newspapers during a ten-year period as well as the themes that were covered more frequently. After a thorough examination of the articles reviewed for the study, it became evident that ethical or moral issues were the most prevalent topics. The study concluded that a more in-depth discussion of the risks and benefits of AI is required for a critical evaluation of the technology's use and regulation (Chuan et al., 2019) .

Survey outreach

Other literature has used a methodological approach that is more individualized to gather information on how the general population perceives AI. Yeh et al. examine the perceived understanding and involvement with AI among Taiwanese survey respondents. Forty-three percent of the 1108 respondents identified themselves as "slightly" understanding AI, according to the study. When asked about their specific involvements with various AI-enabled devices and applications, however, over fifty-seven percent of respondents reported a moderate to high level of familiarity with the technology (Yeh et al., 2021). This accentuates public oblivion in nations abroad. The study presented in this paper adds to the discussion by characterizing public perception and sentiment on AI with a primary goal of providing a foundation for conducting similar empirical research in the future.

Methods

A survey containing likert assessments and affective questions was used to collect data regarding the overall impact of AI as well as its impact in three specific high-risk domains. A high-risk domain is an application area where the ethics of the technology's use are questioned due to unintended consequences. The job equality domain refers to the fairness in obtaining and keeping an easily automatable job. The user behavior domain describes the use of technology to influence user behavior, typically for monetary gain. The decision-making domain refers to the method of synthesizing large amounts of data to automate the process of making significant decisions. When investigating public opinion and attitude regarding AI, these three domains are of primary interest.

Data collection

The survey firm Survey Monkey was contracted to administer the questionnaire to American adults in the United States. Respondents were able to fill out the survey hosted on the platform using a computer or mobile device. There were over 280 valid survey responses to our survey.

Survey Questionnaire

Respondents were asked about the influence of AI on humanity in general as well as on three distinct domains: job equality, user behavior, and decision-making. The global impact of AI was assessed by a scale that asked: "How would you assess the overall impact of AI on humankind?". Similar wording was used to assess the perceived impact of the three domain-specific AI applications. Respondents answered using a five-point likert scale, ranging from 1 (extremely negative) to 5 (extremely positive). For each

domain, respondents were also asked to rate their level of agreement on a five-point likert scale, ranging from 1 (strongly disagree) to 5 (strongly agree), on four different statements that were equally distributed as either a benefit or concern of the application of AI in that domain. Statements were formulated to emphasize either a positive or negative outcome resulting from the implementation of AI in the specified domain.

Following each domain-specific general likert assessment and matrix-style question, survey respondents were also asked to answer an affective question about the impact that AI has on each domain-specific application, expressing how they feel about it. In particular, respondents were asked, "How do you feel about the impact of AI on employment opportunities for the general population?". Modifications were made to the query's language to accommodate the relevant domain context.

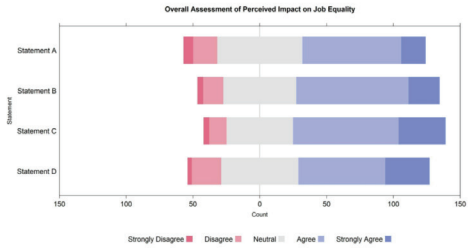
Results

Perceived impact of AI on humankind and within three domains.

The data collected reveals a hopeful tone for the receptivity of AI. The majority of respondents had a neutral or favorable opinion of the technology based on their responses to the likert scales that assessed the overall impact that AI was perceived to have on humanity and the three specific domains. Many respondents rated the overall impact of AI on humanity and across the three domains as positive or extremely positive (38.9%). Forty and three hundredths of a percent deemed the overall impact of AI on humanity and the three domains to be neutral (40.3%). Twenty and eight hundredths percent of respondents rated the overall impact of AI on humanity and across the three domains as negative or extremely negative (20.8%).

FIGURE 1:

Proportions of perceived impact that AI has on humankind generally and across three specific domains.

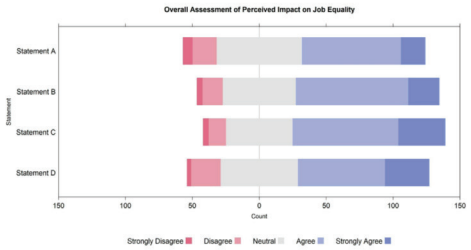


Risk-benefit assessment in the job equality domain.

The level of respondents' agreement with four statements formulated and distributed equally as either a benefit or a concern in each domain was analyzed using matrix-style questions. Over half of respondents agreed or strongly agreed with the benefit statements for the job equality domain (55%). Many responses were neutral regarding the benefit statements (32.8%), while a small percentage disagreed or strongly disagreed with them (12.2%). Similar tendencies can be observed in the level of agreement with the concern statements. More than half of respondents (58.4%) expressed agreement or strong agreement with the concern statements. Plenty indicated a neutral stance on the concern statements (29.8%), while a minority disagreed or strongly disagreed (11.7%).

FIGURE 2:

Proportions of perceived impact of AI on job equality through assessment of benefits and concerns (Statements A & B are related to benefits. Statements C & D are related to concerns).

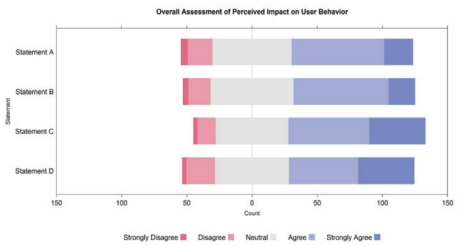


Risk-benefit assessment in the user behavior domain.

In the user behavior domain, slightly over half of the respondents agreed or strongly agreed with the benefits statements (52.2%). Many responses indicated a neutral stance on the benefits statements (35.1%), while a minority indicated disagreement or strong disagreement (12.7%). More than half of the respondents either agreed or strongly agreed with the concern statements. A sizable proportion of respondents held a neutral stance on the concern statements (31.7%), while a minority disagreed or strongly disagreed with the concern statements (11.8%).

FIGURE 3:

Proportions of perceived impact of AI on user behavior through assessment of benefits and concerns (Statements A & B are related to benefits. Statements C & D are related to concerns).

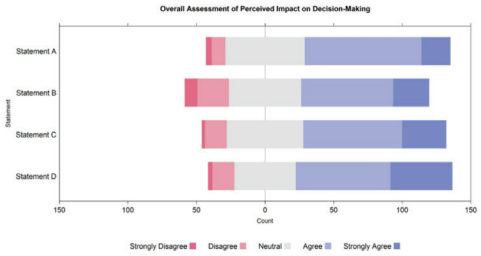


Risk-benefit assessment in decision-making domain.

For the domain of decision-making, the same trends observed in the ranking of benefit and concern statements in previous domains are observed. Over half of the responses indicated they agreed or strongly agreed with benefit statements (55.9%). A sizable proportion of respondents expressed a neutral stance on the benefit statements (31.2%), while a minority of responses indicated they disagreed or strongly disagreed with the benefit statements (12.9%). The overwhelming majority of respondents agreed or strongly agreed with the concern statements (61.2%). Many responses held a neutral stance on the concern statements (28.4%), while a minority disagreed or strongly disagreed (10.4%).

FIGURE 4:

Proportions of perceived impact of AI on decision-making through assessment of benefits and concerns (Statements A & B are related to benefits. Statements C & D are related to concerns).



Text mining for sentiment analysis on perceived impact of AI for all domains.

For a more rigorous analysis of the two types of likert assessments across all domains, affective question response data was collected for each domain. After reviewing each matrix-style evaluation of benefits and concerns, survey respondents were able to express their feelings regarding AI's impact on a specific domain. Using techniques for sentiment analysis in the programming language R, the results indicate that the majority of respondents viewed the impact of AI positively across all domains. Sixty percent of text responses contributed to a positive sentiment.

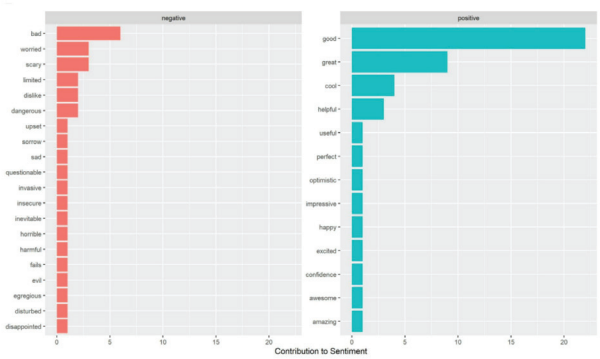
FIGURE 4:

The relative importance of specific sentiment keywords as represented by font size.

While there was a wider range of keywords used to describe "negative" sentiments, "positive" words were more abundant from the data.

FIGURE 5:

The distribution of negative (red) and positive (blue) keywords used to discover overall sentiment trends.



Discussion

While the majority of respondents expressed a neutral, positive, or extremely positive assessment of perceived impact that AI has on humanity and within the three domains, the matrix-assessments for each domain reveal a conflicting representation on the perception of AI. The majority of respondents concur with both the benefit and concern statements for each domain. Respondents accept both the positive and negative aspects of domain-specific AI applications, rather than agreeing with one side or the other. However, further investigation reveals that emotional evaluation questions across all domains tend to have a positive sentiment. Although it is impossible to draw a definitive conclusion from this study, the data is consistent with previous research in that it indicates a general decline in existentialist beliefs.

In addition, we develop a heuristic for evaluating public opinion on related topics and suggest future research areas based on the findings of this study.

A multidimensional and relational perspective with a keen focus on context.

According to the findings, the general public perceives that AI has a good influence on humanity and within three distinct categories, however most respondents equally accept the advantages and risks of each application's unique domain. This inference shows a favorable attitude toward the technology but also points to skepticism about certain applications of AI.

Parsing the matrix assessment data reveals potential justifications for the polarity. By choosing to focus on respondent answer trends for benefits and concerns across all domain-specific applications, new dimensions behind the problem are explored. Contextualizing benefit and concern assessments is critical to understanding why survey results indicate an equal level of agreement with risks and benefits associated with domain-specific AI applications. We hypothesize that certain domain-specific applications will appeal more to specific individuals. This can be driven by the rationale that respondent exposure to AI technology is context-dependent. Given that persons of any age 18 and over were eligible to participate in the poll, it is important to take into account the potential disparities in technology use among young and older respondents. While no attempt was made to decode the technological generation gap in this

study, a number of studies have linked the current generation to increased technology use (Vogels, 2019). A younger respondent may have a greater likelihood of being exposed to AI-enabled technology than an older one who has no need for it. Furthermore, a subset of individuals may opt to consume sensationalized media coverage of AI, which may exacerbate the opinion polarity.

Conducting correlational research to determine significant attitude predictors.

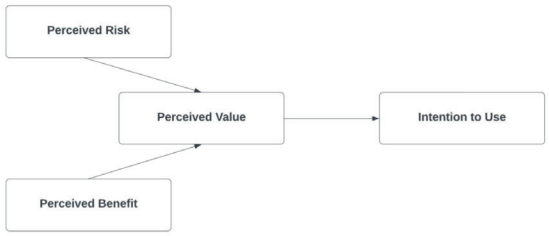
The findings of this study could be improved by evaluating background factors. Taking into account variables such as domain-specific experience, technological exposure, and subscription to major news channels, it may be possible to gain a broader perspective on some domain-specific applications of AI. Reframing the methodological approach by questioning about the respondent's history can enhance our understanding of public receptivity to AI. In essence, it would provide a more holistic perspective that would assist guide developers and legislators more accurately.

Establishing a heuristic for future discussion.

The findings contribute to the discussion and provide useful information that might influence future research. More importantly, the methodology will give a solid baseline for undertaking comparable empirical research. A risk-benefit analysis is a valuable instrument for gauging public acceptance and support for a certain technology. The experimental heuristic outlined below could aid researchers in determining whether AI applications are losing public acceptance.

FIGURE 3:

A flowchart representing a risk-benefit framework for conducting perception inquiry.



When doing comparable perception research, researchers may use this graphic to inform their methodological approach. In an experiment, participants may be asked about their opinions on a certain technology, for instance. In an effort to promote or regulate a technology, it is possible to assess if the benefits outweigh the risks by comparing the risks and benefits associated with the technology. This approach to empirical research proves to be concrete and quantifiable, serving as a consistent indicator for perceived product value.

Conclusion

In this study, we present the results of a survey of 280 individuals regarding how they see the influence of artificial intelligence in both general and domain-specific applications. While the majority of respondents in the survey have a favorable view toward AI, there is a similar degree of consensus about the advantages and disadvantages of domain-specific applications. Consistent with past findings, the results of the sentiment analysis help to underscore the positive acceptance of artificial intelligence. To comprehend the perceptual landscape of AI technology, further contextual study is required.

Understanding how the public perceives artificial intelligence is crucial for product creation, research, and public policy. Therefore, a feedback loop is essential in field research and development. To control the development of artificial intelligence effectively, the general public must be a stakeholder in the technology, and academia must be prepared to bridge the gap between public opinion and policy. The purpose of the present study is to characterize how the American public now views AI. Importantly, the study closes with a paradigm that, when applied to available research on the topic, can potentially estimate relative product value. The purpose of the study is to improve the design of future research and provide information on artificial intelligence deployment areas where public support is waning. When these results are considered collectively, relevant stakeholders may operationalize governance in AI-enabled applications lacking a robust human factors approach.

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Christian Flores

McNair Cohort: 2022

Biography:

My name is Christian Flores. I am a recent 2022 graduate with a bachelor's degree in Cognitive Science. During my time at UCSD, I entertained the practical and theoretical aspects of my major. I recently completed a self-guided research project that explored the social impact of artificial intelligence. My research interests generally lie at the intersection of computing and social impact. I will pursue a masters degree in Computer Science beginning in the Fall of 2023. My plan is to obtain a PhD in Computer Science to pursue a career in academia.

Acknowledgements:

I would like to thank my mentor, Dr. Sean Kross for his invaluable advice in completing my research paper. I would also like to thank the wonderful staff in the Undergraduate Research Hub and within the McNair Scholars Program for providing me with relentless support and guidance throughout the research process.

> ***"I was motivated to engage in research by a curiosity to explore beyond the proficiency and application I gained from regular coursework, seeking deeper insights into a relevant domain."***

STUDENT **PAPER** 2

Use of CRISPR/Cas9 Gene Editing Methods to Investigate the Mechanism of Trem2-Dependent Gene Expression in Macrophages

Researcher: Mohnish Alishala | Mentors: Thomas A Prohaska & Christopher K Glass

ABSTRACT

Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) is a surface receptor expressed in macrophages during tissue injury. This receptor plays a role in driving phagocytosis and dampening inflammation. Because of this, it plays a large part in diseases such as Alzheimer's disease, liver fibrosis, and metabolic syndrome. Each of these diseases all have a population of TREM2-expressing macrophages that does not exist in healthy tissue. However, the exact pathway in which TREM2 is involved in these diseases is rather unknown. Macrophage gene expression is regulated by a variety of transcription factors such as ATF3 and TFEB. These transcription factors have been suggested to be involved in some of the disease processes mentioned above by RNA-seq or ChIP-seq experiments.

The research question we addressed was how these two transcription factors directly affect transcription in macrophages, specifically in the TREM2 pathway. CRISPR/Cas9 gene editing was used to generate loss of function alleles for each transcription factor. RNA-seq was then used to compare gene expression to define the gene-specific transcriptional roles of each factor and determine whether they play roles downstream of TREM2 signaling.

Results showed that Atf3 knockout had very few genes upregulated or downregulated in the RNA seq compared to Trem2 knockout. Tfeb, on the other hand, had 13 genes in common with Trem2 knockout that were expressed lower than the control and 10 genes in common expressed higher than the control. The Tfeb knockout had no difference in Trem2 expression between the knockout population and control, further providing evidence that Tfeb is located downstream of Trem2. Because Trem2 levels stayed consistent in the Tfeb KO, it is likely that some of the effects of Trem2 on the macrophage disease population genes are directly mediated through Tfeb.

INTRODUCTION

The immune system has a crucial role in protecting and healing the body when fending against pathogenic organisms. Specifically, macrophages are a key part of the first line response of the immune system through phagocytosis of bacteria or infected cells and by activating the inflammatory cascade. They not only provide protection against foreign entities but also assist other immune cells in the healing process. They help the body stabilize homeostasis and aid in tissue repair (Troutman et al., 2021). In addition, they have been described as regulators of tissue repair, regeneration, and fibrosis in multiple other

disease contexts (Wynn and Vannella, 2016). Because of their role in these specific functions of the immune system, they have been declared as potential therapeutic targets, which is why there has been an increased level of research interest surrounding this topic (Wynn and Vannella, 2016).

Several macrophage phenotypes are known to be found in diseased tissues; however, there is only a limited understanding of what exactly causes the diversification of macrophages to result in these diseased phenotypes (Seidman et al., 2020). Seidman et al. delved into understanding how environmental signals related to diseases modify macrophage gene expression in a nonalcoholic fatty liver disease (NASH) mouse model. They identified a substantial increase in the expression of Triggering Receptor Expressed on Myeloid Cells 2 (Trem2) in Kupffer Cells in the NASH model, a gene that is only minimally expressed in Kupffer Cells of healthy livers (Seidman et al., 2020). The NASH diet resulted in more than 800 genes in Kupffer Cells being differentially expressed, showing how environmental signals have a profound impact on macrophage gene expression (Seidman et al., 2020).

TREM2 in particular is a type of surface receptor typically expressed in a subset of macrophages during different types of tissue injury (Gratuze et al., 2018). This receptor plays a role in driving phagocytosis and lipid catabolism, and it was also found to be pushing the remodeling of immune cells on the tissue level (Jaitin et al., 2019). The TREM2 pathway seems to be involved in sensing tissue damage and restricting its spread (Deczkowska et al., 2020). TREM2 has been identified as playing a major role in cell types such as microglia in Alzheimer's disease, Kupffer cells in nonalcoholic steatohepatitis (NASH), and adipose tissue macrophages in obesity (Troutman et al., 2021). The goal of this research is to better understand the transcriptional pathways in macrophages and relate them to transcriptional changes conferred by TREM2. This may provide information to potentially target the transcriptional regulation of macrophages in human disease. Nonalcoholic steatohepatitis, obesity, atherosclerosis, and Alzheimer's disease all have a population of TREM2-expressing macrophages that do not exist in healthy tissue (Troutman et al., 2021). These populations are named disease-associated macrophages (DAM) in Alzheimer's disease, lipid-associated macrophages (LAM) in obesity, and scar-associated macrophages (SAM) in fibrosis (Troutman et al., 2021). Based on knockout experiments, TREM2 has been identified as a key regulator of these populations (Troutman et al., 2021).

However, how TREM2 modulates gene expression is unknown (Xiong et al., 2019). TREM2 binds to a large variety of ligands, including phospholipids, lipoproteins, and apoptotic cells (Deczkowska et al., 2020). Pharmacologically, TREM2 may not be the best place to target therapy because in these disease contexts there are a lot of ligands already present that TREM2 is a receptor for, so it may be better to target something downstream and activate it for less system wide effects and more specificity. Macrophage gene expression is known to be regulated by a variety of transcription factors, including ATF3, EGR2, and members of the MiT/TFE family (Troutman et al., 2021). These transcription factors have been suggested to be involved in some of the disease processes mentioned above by RNA-seq or ChIP-seq experiments (Seidmann et al., 2020, Troutman et al., 2021).

The MiT transcription factors are known to be related to inflammatory and immune responses. Specifically, they have large roles in autophagy, lysosomal biogenesis, and lipid catabolism (Irazoqui 2020). The MiT/TFE transcription family consists of MITF, TFEB, TFEC, and TFE3. Because of their role in inflammatory responses and innate immunity, it makes them strong candidates to be connected with the TREM2 functional pathway as well as possible places of attack for several diseases (Irazoqui 2020). When the NASH diet was introduced to mice, the transcription factor ATF3, from the ATF/AP-1 family, increased in expression followed by TREM2 upregulation (Troutman et al., 2021). This makes ATF3 a good candidate to test in this experiment because of its connection to the TREM2 pathway.

The research question we addressed is how these transcription factors, ATF3 and TFEB, directly affect transcription in macrophages, specifically how these transcription factors are related to the TREM2 pathway. Our Lab defined TREM2 dependent enhancers using epigenetic methods and using computational methods identified an enrichment of motifs for ATF3 and members of the MiT/TFE family of transcription factors. These previous results led to the hypothesis that ATF3 and TFEB (a member of the MiT/TFE Family) are located downstream of TREM2 signaling.

FIGURE 0:

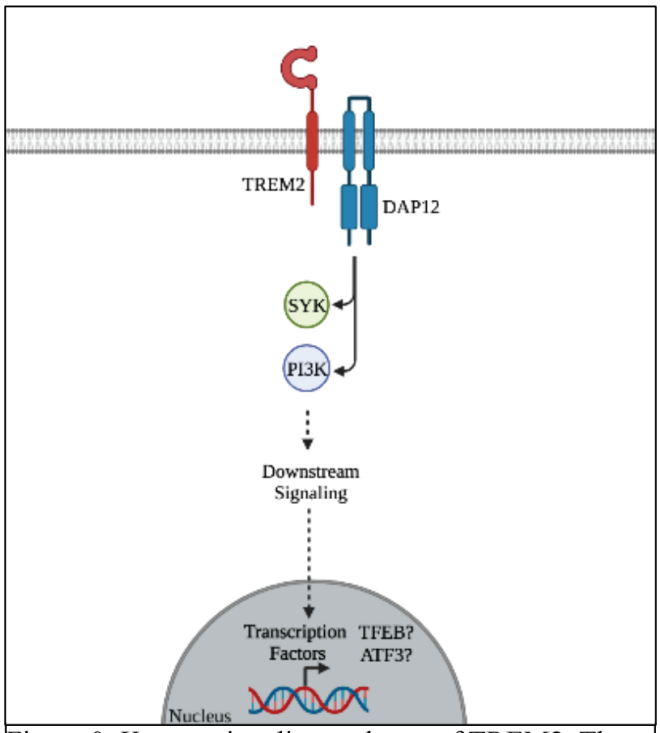


Figure 0: Known signaling pathway of TREM2. The hypothesis was that TFEB and ATF3 were potential transcription factors downstream that TREM2 uses to modulate gene expression.

Adapted from Dezykowska, Weiner, and Amit, Cell 2020.

Created with BioRender.com

Figure 0 shows the known pathway of TREM2 along with where ATf3 and TFEB potentially fit in the pathway. To test this hypothesis, we looked for gene expression overlap between CRISPR knock outs of these transcription factors and the knockout of TREM2 itself in a cultured macrophage model. This hypothesis was tested by using CRISPR/Cas9 gene editing to generate loss of function mutations for each transcription factor in a model macrophage cell line. FACS sorting was used to isolate single cells for clonal expansion, allowing the identification of cells with homozygous frameshift mutations resulting in complete loss of function. RNA-seq was then used to compare the gene expression of wild type and mutant cells to define how they affect gene expression. We then compared these generated knockout cells to the loss of TREM2 in the same cell type, a dataset already created by our laboratory. By using CRISPR, FACS sorting, and RNA-seq, we conducted a comprehensive study of how these factors exert their effect on macrophage gene expression.

Methods

First, gRNA sequences were incorporated into plasmids for cloning. CHOP CHOP (https://chopchop.cbu.uib.no) was used to determine the optimal guide RNA sequence to generate primers for cloning.

Annealing of primers for gRNA was done at 50 µM each of reverse and forward primer in 11xDuplex buffer (1.1M Potassium Acetate, 300mM Hepes), starting at 95°C for 5 min, followed by a decrease in temperature to 25°C at a rate of 5°C/min. BsmBI-digested LentiGuide-mCherry and the annealed primers were ligated by T4 DNA ligase at 16°C overnight.

The ligation mixture and Stbl2 competent cells were incubated on ice followed by a 30 second 42°C heat shock. Bacteria were cultured in SOC media and then plated on an LB agar plate with ampicillin at 37°C overnight.

Each colony grown on the plates was extracted and cultured in LB media with ampicillin overnight at 37°C while shaking at 250RPM. DNA was then extracted from the cultured colonies using the Zymo Mini Prep purification kit. DNA was sent for Sanger sequencing to confirm the

insertion of the correct gRNA sequence into the plasmid.

Once the correct sequence was confirmed, 100mL LB (Amp+) cultures of the transformed bacteria were incubated overnight in incubator (250 RPM, 37°C). Then, Macherey-Nagel Midi Prep DNA purification kit was completed to procure 5 µg of plasmid DNA.

293T cells were hawed and then cultured in DMEM10 which includes DMEM with glutamate, penicillin, streptomycin, and 10% fetal bovine serum (FBS) until 70-80% confluency was reached. The cells were trypsiniszed and counted to add ~3.5 x 106 293T cells into every 10 cm dish with DMEM10 as media.

The media was changed to DMEM supplemented as above, except with 30% FBS. 5µg of the lentiviral vector, 3.75µg of psPAX2, and 1.25µg of pVSVG along with XtremeGene HP was mixed in Opti-MEM media and then added to the 293T cells in the 10 cm plates. 24 hours and 48 hours after lentiviral vector was added, supernatant media containing the virus was collected and filtered through 0.45 µm filters. 10µg/mL fibronectin was added to a 12 well plate and placed in an incubator for 1 hour (37°C).

Cas9-expressing conditionally immortalized macrophages (ER HoxB8 cells, generated as described in Shen et al., eLife 2022) were cultured in RPMI media with glutamate, penicillin/streptomycin, 10% FBS, 20ng/mL GM-CSF, and 0.5µM β-estradiol to prevent differentiation. LentiBlast and polybrene were added to each well along with 500,000 cells in RPMI. Finally, the virus or DMEM for the negative control well was added. Transduction was carried out during centrifugation at 1000g for 90 minutes at room temperature. After 5 days, successfully transduced cells (mCherry positive) were single-cell sorted by FACS into 96-well plates.

Of the grown clones of cells, DNA was isolated through the Qiagen Nucleic Acid Extraction Kit. PCR was conducted to amplify the genomic region targeted with gRNA and gel electrophoresis was run with the PCR product. DNA was extracted from the gel, purified, and Sanger sequenced to assess for clones with homozygous frameshift mutations on both alleles.

Clones with homozygous frameshift mutations (as well as clones with control gRNA) were differentiated into macrophages. Cells were washed twice with PBS, followed by differentiation in DMEM with 17 ng/ml M-CSF for 7 days. Cells were lysed in TRIzol Reagent and RNA was isolated. The RNA was fragmented, reverse transcribed to cDNA, and then the libraries were prepared and amplified. Finally, it was sequenced on a NextSeq 500 or Hi-Seq 4000 (Illumina, San Diego, California). It was performed as done in Cobo et al. RNA-seq analysis was completed through the HOMER pipeline to better understand the gene expression of wild type and mutant cells (Heinz et al. 2010).

Results

RNA seq analysis was done through the HOMER pipeline and it was used to calculate the log2 Fold Change and the p adjusted values (Heinz et al. 2010). These two values were then used to determine which genes in the knockout cells were significantly upregulated and downregulated. For a gene to be considered significantly upregulated or downregulated, it required a 1.5 times difference in transcripts per million when comparing the control to the knockout population and a p adjusted value of less than 0.05. The red dots in Figures 1 and 3 represent significant upregulation and the blue dots represent significant downregulation.

FIGURE 1:

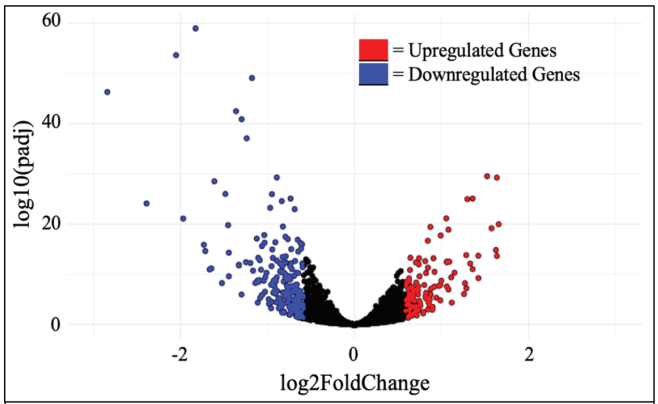
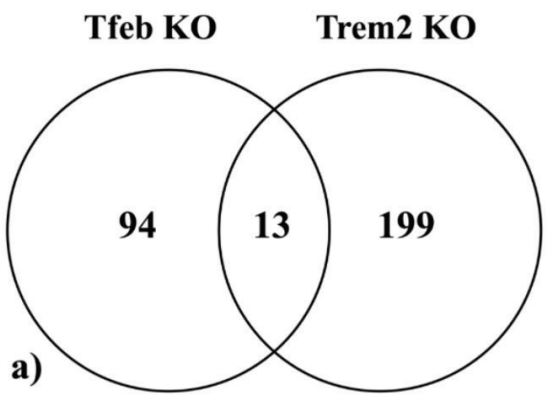


Figure 1: Volcano Plot of RNA seq results of Tfeb KO cells vs. control. Upregulation and downregulation for genes were determined by p adj < 0.5 and Fold change > 1.5 or < -1.5.

FIGURE 2:



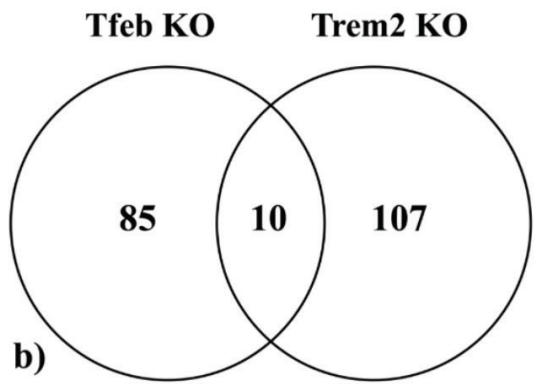


Figure 2: There are several genes regulated in common between the Tfeb KO and Trem2 KO compared to the control.

- Venn Diagram of downregulated genes
- Venn Diagram of upregulated genes

For Tfeb KO, Figure 1 shows there were several genes that were both upregulated and downregulated. Figure 2a identifies 13 genes in common that were downregulated in both populations of knockout cells compared to the control. These 13 genes were Cx3cl1, Gadd45g, Tmem132a, Zfp503, Pparg, Gngt2, Slc15a3, At3, Cd9, Tgm2, Sdc3, Plekho2, and Gnas. There were 10 genes in common that were upregulated in both populations compared to the control (Figure 2b). The 10 genes were Ccnb2, Cenpa, Ccl2, Mtus1, Ncapg2, Nt5dc2, Slc16a6, D17H6S56E-5, Egr1, and Rhob.

FIGURE 3:

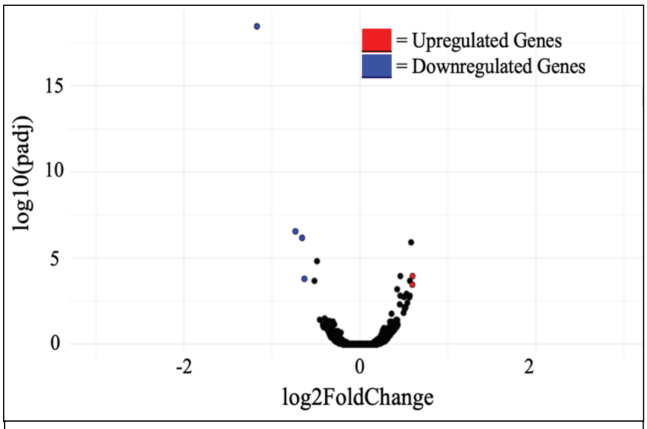


Figure 3: Volcano Plot of RNA seq results of control vs. Atf3 KO cells. Upregulation and downregulation for genes were determined by p adj < 0.5 or Fold change > 1.5 and < -1.5.

FIGURE 4:

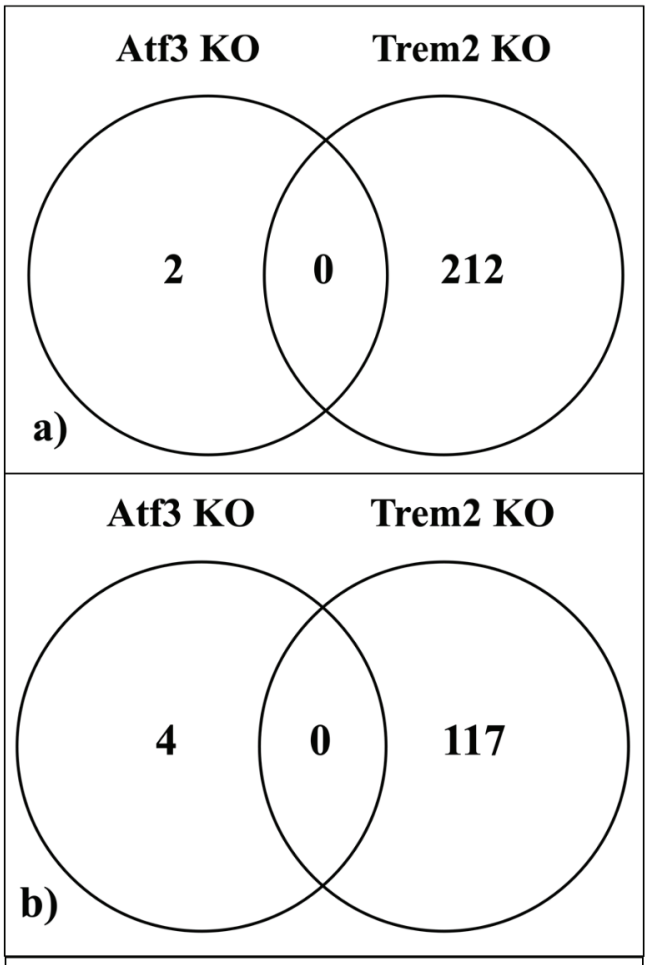


Figure 4: There are no genes regulated in common between the Atf3 KO and Trem2 KO compared to the control.

- Venn Diagram of downregulated genes
- Venn Diagram of upregulated genes

For Atf3 KO, Figure 3 has very few genes that were significantly upregulated and downregulated. The venn diagrams in Figure 4 reveals that there were zero genes upregulated in common between Atf3 KO and Trem2 KO.

FIGURE 5:

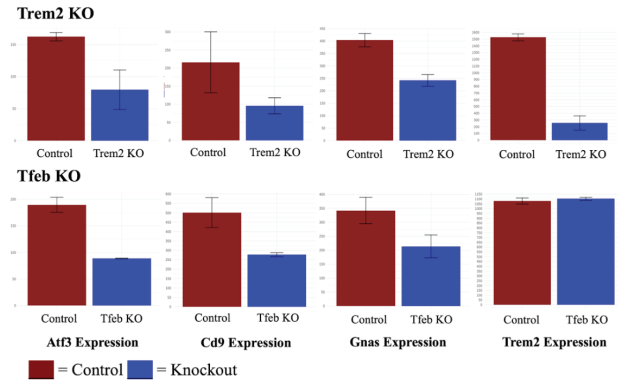


Figure 5: Bar plots comparing gene expression of select genes between the knockout population and the control population.Y-axis represents transcripts per million.

The genes chosen in Figure 5 are all signature genes of the earlier mentioned DAMs, LAMs and SAMs macrophages from the Troutman et al. literature review. The first row of plots of Figure 5 are from the Trem2 KO cells while the second row of plots is from the Tfeb KO cells.

Previous research showed there were 6 genes that were downregulated in these DAM/LAM/SAM populations as well as Trem2 KO when comparing to the control (Prohaska 2022). In Tfeb KO, Atf3, Cd9, and Gnas, 3 of the 6 genes, were found downregulated as well. For the first three sets of plots in Figure 5 from the left to the right, the gene expression profile is similar where the knockout of the gene in question resulted in a decrease in gene expression compared to the control. In the fourth set of plots, the Trem2 KO resulted in a decreased expression of Trem2 in the cells. However, the knockout of Tfeb results in no significant change in Trem2 expression when comparing the control and the Tfeb KO cells.

Discussion

The results support the potential role of Tfeb in downstream Trem2 signaling, but not Atf3. Because Trem2 levels stayed consistent in the Tfeb KO, it is likely at least some of the effects of Trem2 on the DAM, LAM, and SAM genes are directly mediated through Tfeb. It further provides evidence that Tfeb is located downstream of Trem2.

For Atf3, there was little significant upregulation and downregulation of genes. Therefore, Atf3 KO needs to be tested in different conditions such as amylin and myeloid to see if there will be a difference in the gene expression after knockout.

These KO experiments were performed in an immortalized cell line in vitro in which the Trem2 pathway is constitutively activated. Previous research has shown that the in vivo environment is very important in maintaining macrophage identity, including induction of the Trem2 pathway itself (Gosselin et al., 2017). Therefore, there may be differences in the consequences of these knockouts in an in vivo setting. The Tfeb and Atf3 KO must be performed in an in vivo setting to compare the generated data from this experiment to better understand these experiments.

In the future, further KO experiments can be conducted with other transcription factors in the MiT/TFE and ATF/AP-1 families. Glass Lab RNA seq data has found that three members of the MiT/TFE family and more than six members of the ATF/AP-1 family are expressed in HoxB8 cells. It is likely that these factors are at least partially redundant, which would result in more modest effects when a single factor is deleted. As a result, more severe phenotypes might be expected if combinations of KOs were generated. Additional downstream experiments could be performed by investigating the consequences of macrophages with knockouts of these transcription factors in specific disease models such as Alzheimer's disease, metabolic syndrome, and liver fibrosis.

All in all, the data discussed here may help uncover the Trem2 pathway so that we may potentially create novel therapies for diseases that are currently challenging to treat.

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Mohnish Alishala

Faculty Mentorship Program: 2021–22

Biography:

My name is Mohnish Alishala and I am a third-year human biology major. At UCSD, I am a head coach for StRIVE, a club where we coach adult students with disabilities to help them transition to independent living. I am also a member of Camp Kesem where we fundraise money throughout the year to create summer camps for children whose parents have cancer. My involvement on campus gives me an opportunity to make a positive impact on other people.My research interests focus on the immune system and how our body fights against illness. This ties in with my career goal of becoming a physician.

> ***"As an aspiring physician, I was interested in pursuing biomedical research to learn more about the immune system from a different perspective."***

Acknowledgements:

I would like to thank my PI Dr. Christopher Glass for his continued support in my research and for my mentor Dr. Thomas Prohaska for always helping me patiently and teaching me valuable lab skills. I would also like to thank the Faculty Mentorship Program for its guidance in this research process.

STUDENT **PAPER** 3

Treading on the Tiger's Tail: Chinese *Wuxia* and Japanese *Jidaigeki* Action Films Reacting to State Censorship in the 1930s and 1940s

Researcher: Zerui Pan | Mentors: Dr. Geraldine Fiss & Dr. Daisuke Miyao

ABSTRACT

This article highlights the relationship between *wuxia* (martial heroes) and *jidaigeki* (period drama) action films and state censorship in the 1930s and 1940s. I first introduce readers to key East-Asian literary conventions that portray righteous warriors who incarnate their moral codes with swords. I then illustrate the political contexts in 1930s China and 1940s Japan which caused the popular film genres of *wuxia* and *jidaigeki* to become politically problematic and therefore strictly censored. I closely examine director Bu Wancang's 1931 *wuxia* film *A Spray of Plum Blossoms* and Kurosawa Akira's *jidaigeki* 1945 film *Treading on the Tiger's Tail*, investigating creative solutions each director found in order to release their respective films despite censorship pressures. There are three inventions both directors pioneered in their work, namely "ostensible exactness" in setting; "patriotic warriors" in characterization; and "swordless fights" in presenting spectacular action scenes. I argue that although these solutions failed to restore both genres back to their former popularity, Kurosawa's film paved the way for the success of his world-class *jidaigeki* in the 1950s, and Bu's *wuxia* led to the emergence of kung fu films in the 1960s. My critical analysis underscores the innovative creativity of Chinese *wuxia* and Japanese *jidaigeki* films while calling attention to the artistic and cultural legacies of these two popular cinematic genres. The comparison demonstrates how film censorship may undermine or even destroy traditions that have a long history and deep cultural roots.

Detouring Censorship: Suppression, Creation, and Adaptation in Chinese *Wuxia* and Japanese *Jidaigeki* Films

Both Chinese *wuxia* and Japanese *jidaigeki* are subgenres of East Asian action cinema that achieved peerless domestic popularity in the late 1920s and early 30s, respectively. They generated success not only by portraying righteous warriors that embodied their moral codes with swords but also by catering to the general public's desire for justice and order in a turbulent time. However, these cinematic genres gradually lost their dominance in the domestic film industry during the 1930s. In 1931, the production of Chinese *wuxia* was prohibited by the Chinese nationalist government for promoting superstition and anarchy. Fearing the leftist elements in *jidaigeki*, the militaristic

government in Japan also began to suppress its production in the mid-1930s, and *jidaigeki* films were strictly censored during the U.S. occupation period (1945-1952).

Director Bu Wancang was a prolific Chinese director and screenwriter fully aware of the political hostility towards *wuxia*. He made substantial thematic and cinematographic changes to his *wuxia* film *A Spray of Plum Blossoms* 一剪梅 (Yi Jian Mei, 1931) so that it might pass state censorship. The renowned Japanese director Kurosawa Akira also adapted a kabuki play into an unconventional "swordless" *jidaigeki The Men Who Tread on the Tiger's Tail* 虎の尾を踏む男達 (Tora No O Wo Fumu Otokotachi, 1945) in order to release the film despite enormous censorship pressure from both the militaristic Japanese government and the U.S. Civil Information and Education Section (CIE). Both directors experimented with creative solutions to circumvent state censorship while keeping the aesthetic values intact and the political message undistorted. In this article, I demonstrate how both filmmakers place the fictional narration in a real historical background to advocate nationalism, change the conventional depiction of combatants in *wuxia* and *jidaigeki* from anti-authoritarian rebels to conforming patriots, and present swordless action scenes to circumvent contemporary censorship laws. Although none of these attempts succeeded in reinstating either genre back to its heyday, I contend that they helped to preserve and innovate the generic conventions of *wuxia* and *jidaigeki*, allowing the two genres to survive suppression and rebirth in the 1950s and 1960s.

Burning of the Sword: The *Wuxia* Tradition and Its Decline

The term "*wuxia*" is a noun consisting of two separate characters "wu" 武 denoting physical combat, and "xia" 侠, referring to morally righteous warrior figures. The initial mention of the term can be traced back to the mid-3rd century BCE (Ye, 1997). The eminent figure of the legalist school of Chinese philosophy, Han Feizi, denounces *xia* for abusing their strength to transgress the law of the state in his eponymous work. Such criticism has "resonated among *wuxia* detractors through the ages," including the Guomindang (GMD) or the Chinese Nationalist Party government (1928-1949) who perceived the genre as subversive and thus hampering the building of a legal society (Teo, 2009, p. 19). In the government's view, warriors in *wuxia* are following their personal understanding of justice, which can inspire the common public to transgress government laws. Ultimately, this intrinsic incongruity contributed to the GMD government's suppression of *wuxia* and its production in the 1930s (Zhang, 1999).

Beginning in the 20th century, filmmakers started to seek inspiration from the *wuxia* tradition, adapting popular *wuxia* novels onto the silver screen (Teo, 2009). They believed that adapting *wuxia* fictional tales into films would undoubtedly succeed since the audience loved to watch "picturized" action scenes. However, traditional criticisms of the genre reappeared. Intellectuals who opposed feudalism and supernaturalism vehemently detested *wuxia* films for their unrealistic depictions of "flying sword combat, escape by stealth and other means of subterfuge…" to satisfy popular tastes and entertain the often-undereducated audience (Kung, 1967, p. 157). The excessive use of special effects in *wuxia* films, therefore, placed *wuxia* in a controversial position when the intelligentsia was endeavoring to diminish the influence of superstitions in China.

Meanwhile, GMD noticed the affective power of *wuxia* films on the general public and began to legislate new laws to regulate their content. After uniting most of the nation in 1927, the nationalist government wanted to seek stability and control. Rather than being insufficiently revolutionary, the problem with these films was that they were overtly rebellious. See, for example, *The Mighty Hero Gan Fengchi* 大侠甘鳳池 (Da Xia Gan Fengchi, 1928). In one scene, the righteous protagonists directly condemn the villain as a "nasty officer" *gouguan* 狗官 to initiate a fight (Yang, 1928, 0:04:44). They further demand the

incompetent officer leave his post since a capable government official should protect the people instead of sacrificing their interests to meet his. This particular scene conveys the political message that commoners have the moral right to rise against corrupted government authorities. In January 1931, the Executive Yuan founded the Film Censorship Committee and finally grasped control of the Chinese film industry (Teo, 2009). Despite its popularity, the committee placed *wuxia* on the top of its list of banned genres while pushing major companies out of future production (Guo, 1934). Most large companies switched to so-called "soft" cinema, emphasizing more the form and aesthetics of a work, rather than content that could be politically controversial. Since then, the *wuxia* genre ceased to be the center of Chinese cinema and became a marginalized cinematic genre.

Double Suppression of Japanese *Jidaigeki*

The term *jidaigeki* or "period drama" has its roots in traditional kabuki performance. It refers to films that depict Japanese society before the Meiji Restoration, in contrast to *gendaigeki* or "modern drama" in which stories take place after 1868. The genre is known for featuring sword-carrying warriors and presenting spectacular sword-fighting scenes. Similar to *wuxia* films, *jidaigeki* reached their heyday in the late 1920s, when Japan was transitioning from feudalism to modernity (Gerow et al., 2012). Japanese people in that era were experiencing overwhelming anxiety as their daily lives were increasingly transformed by ongoing modernization (Yoshimoto, 2022). The introduction of monopoly capitalism, for instance, altered Japanese society fundamentally by placing the merchant class over the samurai class in the social hierarchy. Such a drastic change engendered a sense of dislocation not only for the samurai but also for the commoners, who used to look upon samurai moral codes as the bedrock of social order. Simultaneously, the ongoing urbanization process pushed millions of villagers to migrate to metropolitan cities, away from the close interpersonal relationships they once enjoyed in rural life. Out of nostalgia and insecurity, they went to theaters to watch sword masters living their traditional ways of life in the idealized Edo Japan (Yoshimoto, 2022).

The militaristic Japanese government, however, did not share the public's zest but regarded *jidaigeki* as a politically problematic genre that undermined the prosperity of the nation. As the Great Depression hit Japan severely in 1930, resulting in countless youth enthusiastically embracing socialism, nationalist government officials did not want *jidaigeki* to inspire more people to join political radicalism, given that the "most innovative *jidaigeki* filmmakers were themselves radical youth" (Yoshimoto, 2022, pp. 220-221). Moreover, the militaristic government prohibited *jidaigeki* for another distinctive reason: it attacked the genre for containing "Anglo-American" elements, by which the censors meant kissing scenes and extensive use of Hollywood-style filming techniques in *jidaigeki*, such as fast editing and close-ups (Hirano, 1994). As the Second Sino-Japanese War broke out in 1937, the militaristic Japanese government further deemed *jidaigeki* not only a problematic genre leaning toward the "Western enemies" but also a useless medium in terms of disseminating propagandist ideas to support the war effort (Yoshimoto, 2022, pp. 222-224). The golden era of *jidaigeki* eventually drew to an end in the mid-1930s.

While the production of *jidaigeki* came to a halt during the war period, many Japanese filmmakers hoped to revive the genre when the Asia-Pacific War was over. Yet this anticipation proved improbable when the U.S. occupation government took over the country and announced its full control of the Japanese film industry in 1945. On September 22, CIE declared its ultimate purpose of "helping reconstruct Japan positively" by regulating the country's film production (Hirano, 1994, p. 37). Since *jidaigeki* was known for presenting feudal Japan and violent sword-fighting scenes, it inevitably challenged CIE's thematic prohibition of "celebrating feudalism," which was based on the understanding of feudalistic sentiments as responsible for ultranationalism and militarism

(Yoshimoto, 2022, p. 223). Simultaneously, the Occupation government deemed sword-fighting scenes, in which the sword connects Shintoism with Japanese nationalism, as hampering the country to embrace a peaceful future. In the indigenous belief system, the sword is a sacred object that followers may offer to *kami* or the god in Shinto rituals (BBC, 2009). This relationship led the American censors to misinterpret *jidaigeki* as employing sword fights to advocate feudalism and militarism. In sum, *jidaigeki* was a scapegoat, suffering a double suppression from both the wartime Japanese government and the Occupation government after Japan's defeat; a suppression that was not based on a rational ground but unreasonable fear (Yoshimoto, 2022).

Treading on the Tiger's Tail: Bu Wancang and His *Wuxia* Film

The creation of the film *A Spray of Plum Blossoms* (1931) inevitably involved making conscious decisions to sidestep censorship laws and enable this *wuxia* adaptation of Shakespeare's comedy *The Two Gentlemen of Verona* to reach the general audience. The initial change director Bu made was placing the story in a real Chinese city—Guangzhou (Bu, 1931, 19:21). Since *wuxia* typically deals with a fictional setting or *jianghu*, this particularity seemingly rules the film out of the genre. Moreover, the city of Guangzhou carries a strong political connotation as it is one of the major military bases for the nationalist government. Here, director Bu linked his film with nationalism so that it would not fall into the derogatory category of "fantasy films" like most *wuxia* films of the period. Had the film staged the story in a mythical mountain or a remote village, it would foreseeably suffer from accusations of promoting superstition or escapism and would likely not have passed the censorship board.

While most *wuxia* films tend to depict rebellious warriors posting threats on social stability, *A Spray of Plum Blossoms* challenges this controversial convention by repainting the male protagonist Valentine as an ambitious graduate from Republic of China Military Academy (00:01:56). This choice creates a firm connection between Valentine and the authority while endorsing his identity as a member of the existing system rather than an external threat. Meanwhile, it explains where Valentine's sophistication in martial arts comes from. To prevent potential attacks, director Bu cannot let Valentine learn combat skills from a mysterious manual as most protagonists in *wuxia* films do. Otherwise, the film may approach the dangerous zone of promoting supernaturalism and superstition. Linking Valentine to GMD's National Army thus imbues the film with a practical meaning as it might encourage young viewers to realize that joining military training is another way to become a martial arts master.

The director also reshapes the characteristics of the loyal and spirited Silvia (Shi Luohua). He presents her with a higher sense of subjectivity and shrewdness, resembling the heroine in many Chinese *wuxia* films depicting *nüxia* or female warriors. The term *xia* is gender-neutral, though it is often translated into "knight-errant." What happened between the 1920s and 1930s was a proliferation of *wuxia* films celebrating valiant female warriors, such as *Red Heroine* 紅俠 (*Hong Xia*, 1929) and *Woman Warrior White Rose* 女俠白玫瑰 (*Nüxia Bai Meigui*, 1929). In one scene, we see how Bu helps to substantiate the argument here. After realizing Proteus (Bai Lede) might be the slanderer, Silvia tricks him into believing that there has always been a real affection between them (Bu, 1931, 1:32:00-33:04). This unexpected expression of love surprises Proteus, causing him to boastfully confess that he is the actual culprit behind Valentine's banishment. Now the secret is revealed. Silvia suddenly changes from a warm smile into a bitter gaze, condemning Proteus as a despicable betrayer (1:32:55). No matter how Proteus endeavors to persuade her to keep the secret, Silvia firmly rejects his apologies and slaps him twice in the face. This inventive scene director Bu added manifests Silvia's subjectivity as the heroine of the story. Her shrewdness, courage, and righteousness all demonstrate that she can meet the standards of an outstanding female

warrior. In this sense, the audience may appreciate how director Bu manages to portray two heroic *xia* without necessarily annoying the censors.

Since swordplay is no longer an option, director Bu employs a realistic style of filming to present action scenes. The fight between Proteus (Bai Lede) and Thurio (Diao Li'ao) is entirely hand-to-hand combat devoid of any special effects (1:34:45–35:32). There is no more teleportation, flying swords, or any of the fascinating tricks normally found in conventional *wuxia* films; instead, we see "down-to-earth" fistfights and wrestling. Bu inserts several close-ups to intensify the combat, worrying that the audience may not appreciate this plain style of performance. Right before the quarrel escalates into a brutal fight, Bu provides a point-of-view shot of Silvia who is witnessing the whole event (1:34:49). From her terrified visage, the audience can instantly sense the tension pervading the atmosphere. When the fight reaches a climax, director Bu plays the same trick again with a POV shot of Proteus, inviting the audience to substitute themselves into his perspective (1:35:19). This time, the audience is playing the role of a fighter directly involved in the ongoing battle. From Proteus's eyes, the audience watch Thurio squarely staring in their direction with furrowed brows and pursed lips. Then he moves closer and closer to the camera until his blurred face occupies the entire frame (1:35:22). Although the scene does not involve any use of weapons, the audience can unmistakably understand that this is a life-and-death fight as intense as one between two sword-carrying warriors. This exemplary action scene proves that a *wuxia* film can present breathtaking action scenes without relying on special effects, providing a potential direction future *wuxia* directors can take when the conventional way of illustrating martial arts skills is prohibited.

Continue on without Rest: Kurosawa Akira's Solutions to Bypass Censorship

While Bu Wanchang decided to bring *wuxia* to contemporary society, Kurosawa Akira chose to place the story in an earlier historical period to bypass censorship. The Men Who Tread on the Tiger's Tail is a film adaptation of a classic kabuki story named *Kanjinchō* 勧進帳. The adaptation is significant as the narrative takes place in the late Heian period (794–1185), which is distinct from many *jidaigeki* that are based in Edo Japan (1603–1867). Although this choice differs from Bu's solution, it serves the same purpose of painting the film with nationalistic implications. Yoshitsune's escape journey as a real historical event evokes a stronger sense of "Japaneseness" in the viewers compared to fictional Edo legends since it comprises a part of Japanese national consciousness. The creation and maintenance of such consciousness require inventing a collective memory and reiterating it so that every citizen shares a collective identity. By celebrating Yoshitsune's legendary escape and his loyal retainers, Kurosawa selected a story that was present in the collective memory of the people of Japan. This concession he made to abide by the Japanese censorship board simultaneously allowed the film to participate in reinforcing the collective identity and contribute to promoting the government's militaristic agenda in the mid-1940s.

Besides the creative strategy regarding setting, Kurosawa adds a slapstick comedian into his adaptation, who shifts the narration from a third-person point of view to a first-person perspective and thus connects the film to nationalistic sentiments. In the original play, viewers are merely spectators who cannot participate in the story nor substitute themselves into any of the characters; they watch the entire story from a detached third-person perspective. They may sympathize with Yoshitsune's party or admire Benkei's quick-wittedness but are unable to feel galvanized and relate the narrative to the

ongoing war. Incorporating the porter into the narrative enhances the narrator's intimate first-person perspective. In this sense, the viewers are now watching the story through the porter's eyes as if they are accompanying Yoshitsune's party at the scene. To reinforce this effect, Kurosawa frequently employs POV shots of the porter, nudging the audience to accept him as the narrator. Meanwhile, the director allows the porter to demonstrate hyperbolic and hilarious facial expressions based on the potential reaction of the audience. He grins, sweats, or sobs at the exact moment when the audience is supposed to demonstrate the desired emotion. He kneels before Benkei to stop him from beating his master Yoshitsune when the audience cannot take this outrageous act anymore (Kurosawa, 1945, 43:04). He externalizes the audience's emotional journey, which again intensifies the illusion that the porter is the narrator. As soon as the audience accepts this illusion, they may ponder what the porter can do to save Yoshitsune's imperiled life. Subsequently, they might begin to deliberate what a commoner can do to save Japan from losing the war. Kurosawa innovated the porter's role to incorporate a propagandistic element into the film, hoping it might accommodate the censors' political demand for mass mobilization.

Because swordplay was prohibited under the U.S. occupation government's reign starting from September 1945, Kurosawa preserved the sword as a key prop, but he ensured that neither physical contact nor actual combat ever occurred. To maintain the intensity of the action scenes, he employed fast cutting when demonstrating the conflicts between Yoshitsune's party and the barrier guards. A rapid cutting of close-ups occurs when Togashi's advisor tries to check whether Benkei is reading from a real subscription list (34:16-27). Within eleven seconds, the film presents nine close-ups of Yoshitsune's retainers and how they react to the advisor's approach. Such a series of shots intensifies the tension in the atmosphere since the advisor may arrest Yoshitsune's party once he realizes that Benkei is reading from a blank scroll. Although the audience is watching mostly facial expressions, the viewer can recognize how imperiled the situation is for Yoshitsune and his men. Simultaneously, the use of fast cutting contrasts other characters with Benkei's composure before potential death, encouraging the audience to again appreciate his heroism. Though this particular scene is as abstract and theatrical as a kabuki dance devoid of any actual combat, it is nothing less intense and thrilling than a conventional sword fight with Benkei nothing less heroic than an authentic warrior. In a word, this cutting technique envisions a swordless form of action scenes, which helps the film abide by the governmental banning of the sword.

Rising From the Ashes: Chinese *Wuxia* and Japanese *Jidaigeki* in the 1950s-60s

Having closely examined the two films, the question that arises is whether the creative solutions each director used helped each genre to survive and remain popular in subsequent decades. The release of *A Spray of Plum Blossoms* in 1931 hardly attracted the GMD censors' attention. It was generally received as an innocuous romance despite its resemblance with another well-known *wuxia* film, *Woman Warrior White Rose* (Bao, 2005). Bu's innovative attempt, however, could not save the *wuxia* genre from declining. As the suppression continued, *wuxia* films disappeared from Chinese cinema between 1933-1938 and remained a marginal genre until 1949. For the next three decades, *wuxia* films were totally banned in mainland China since the film industry was being nationalized into a political tool for promoting communism (Chen, 2005). As a result, Bu Wancang moved to Hong Kong in 1948 along with many other *wuxia* directors. Their arrival coincided with the early exploration of a new action genre, kung fu. In a way, director Bu's experimental representation of action scenes in *A Spray of Plum Blossoms* established a distinct set of aesthetic standards free from extensive special effects, which

contributed to the development of the new genre. Kung fu distinguishes itself from *wuxia* due to its emphasis on "real fighting" while inheriting the righteous nature and rebellious spirit of *wuxia* (Teo, 2009, p. 58). The rise of kung fu films in the 1960s Hong Kong and their reception by the international audience, therefore, demonstrates the vitality and resilience of the *wuxia* tradition. Even though this tradition of celebrating righteous warriors has been suffering from criticism and censorship since the beginning, it can always adapt to various political environments and continue to play a significant part in defining Chinese culture.

On the other hand, the prohibition of Kurosawa's *Treading on the Tiger's Tail* persisted until 1952, seven years after its production. Even though Kurosawa endeavored to sidestep political concerns, the film still received strong criticism from Japanese censors for interrogating the feudal tradition instead of supporting it and from American censors for "excessively promot[ing] old-fashioned Japanese values" (Watson, 2020). Due to this double suppression, Kurosawa edited the film several times and the extant version of this originally feature-length film is only 59 minutes long. Ultimately, his artistic inventions failed to facilitate the film to overthrow the suppression of *jidaigeki*. The radical reconceptualization of modern Japanese history also demanded postwar Japanese cinema to concentrate on the "immediate past and contemporary chaos," which further accelerated the decline of the historical genre (Yoshimoto, 2022).

Nevertheless, the creative solutions Kurosawa used to circumvent censorship paved the way for his later more successful *jidaigeki* such as Rashomon 羅生門 (1950) and *Seven Samurai* 七人の侍 (*Shichinin No Samurai*, 1954). The prohibition of filming *jidaigeki* got lifted after the U.S. occupation government handed over the control of the country back to the Japanese people in the early 1950s. Nonetheless, the experience of copying censorship triggered Kurosawa to reexamine this genre, realizing that a first-class action film should not only portray grand actions but also human psychology (Yoshimoto, 2022). In *Seven Samurai*, for instance, he used tracking shots to present the breath-taking duel between the sword master Kyuzo and his challenger (Kurosawa, 1954, 48:42-49:18). The camera stays static when the two swordsmen are confronting but starts to shift horizontally following the back and forth of their strikes. The tracking shots allow the audience to watch the fast-rhythm duel as if they are at the scene, which in turn engages the audience to comprehend the agitation of the challenger and the composure of the master. This combination of spectacular actions and deep human psychology became an indelible trademark of Kurosawa's *jidaigeki*, which won him and the genre recognition on the world stage in the 1950s.

With their ingenuity and courage, Bu Wancang and Kurosawa Akira saved the *wuxia* and samurai tradition from state suppression, preserving the integrity of the East-Asian cultural heritage. *Wuxia* and *jidaigeki* films today are still recounting legends of righteous warriors waving their swords to oppose mighty oppressors, although the generic conventions have been changed while the content of the stories altered. The comparison is essential to illustrate that film censorship has the potential to distort or even destroy cultural traditions despite their differences in political or social climate, in this case, the warrior tradition in China and Japan. Even though *wuxia* and *jidaigeki* did not share the same historical context, they could not escape from political suppression and thus had to amend themselves to abide by censorship rules. Truly, Bu and Kurosawa are heroes who dared to tread on the tiger's tail and resembled the *wuxia* and samurai spirit of undauntedly opposing unjust treatment under threats and pressure.

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Zerui Pan

TRELS Cohort: 2022

Biography:

I'm a senior student majoring in world literature and culture, minoring in Japanese studies. I'm also a contributing writer in the Prospect Journal at UCSD. In my spare time, I enjoy reading, cross country running, and snowboarding. My future goal is to become a scholar in comparative literature, facilitating intercultural communications that are essential in today's world. I'm currently researching on East-Asian literature and cinema and I hope my work will help to represent the underrepresented Asian American community in California.

"Diversity is essential to happiness, and in Utopia there is hardly any. -- Bertrand Russell"

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ALUMNI
SPOTLIGHT

Interviewed by
Iliana Kleiner

Cindy Barrientos

Class of 2016 | B.S. in Physiology and Neuroscience & M.S. in Biology
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Graduated in 2016 with a Bachelor's degree in Physiology and Neuroscience along with a Master's degree in Biology, Cindy Barrientos has always loved learning. As a child, she spent her time reading to expand her knowledge. She expressed that she often "romanticized the idea of being the first person to discover something." Ultimately, this aspiration led her to pursuing research as a college student.

She entered UC San Diego as a transfer student; thus, she wanted to be involved in a program that would allow her to make the most of her time at the university. After receiving her acceptance to the school, Barrientos applied to all the programs she was eligible for. Her acceptance into the McNair program– a program within the Undergraduate Research Hub (URH)–landed her a summer research position for the summer of 2014. She continued this research throughout her undergraduate career. This research was interested in how cocaine affects dendritic spine density within certain areas of the brain and how that correlates with addiction.

Following that summer, she continued working with the lab where she ultimately published her research for her Master's degree. Although her research didn't influence her career path, she was able to learn many transferable skills, such as being able to parse through research papers and critical thinking.

However, Barrientos did not always know she wanted to pursue a graduate degree. Not having any family members who worked in the STEM field, she did not have a plan for her future. However, the contiguous BS/MS program she was involved in pushed her to complete an additional year of school to get a graduate degree and help her get into a highly-regarded Ph.D. program, should she choose to pursue one. Barrientos is thankful for her degrees and time spent at school.

After graduating from UC San Diego, Barrientos worked at Irvine Scientific on their New Technologies Team. She helped the company develop new products it wanted to bring to the market. Through friends who also worked in the field, Barrientos learned that "in tech, the sky is the limit. You can learn anything that you want to do on your own time; all of the resources are out there." However, while she loved working on this team, she realized that she did not want to work in the BioTech industry in the long run. She "felt that doing BioTech was not really feasible to be learning quite as much or as frequently as [she] would like."

As a result, she navigated into working as a Data Consultant for RackSpace Technology, a cloud computing company. Barrientos explained that she loves this job because it allows her to do both technical work and interact with her clientele. As she reflects on her time at UC San Diego, Barrientos wishes that she found a better balance between her academic and social lives; she explained that as a student, she was academically and research-oriented, and therefore would have tried to have more fun as a young adult.

One piece of advice that Barrientos would give to students who are curious about doing research on campus is to find a graduate student or professor who may be willing to mentor them. She hopes that students find scientists who inspire them to get more involved in on-campus research and stay motivated while working. Most importantly, Barrientos wants students to understand that "there's a lot of things that can and will go wrong, but just don't forget that the research that you're doing and the discoveries you're uncovering could potentially save lives in the future. It's not negligible work."

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