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UNIVERSITY OF CALIFORNIA, RIVERSIDE

UNDERGRADUATE RESEARCH JOURNAL

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FROM THE CHANCELLOR

As a research university, one of UC Riverside's most important duties is the creation of knowledge. Undergraduate research is a hallmark of UCR's scholarly and educational missions and a factor in our university's impressive trajectory.

With faculty-mentored research projects across a breadth of disciplines, UCR provides a wealth of opportunities for students to investigate complex questions and discover the joys of scholarly research.

As you will see in this 17th volume of the *UC Riverside Undergraduate Research Journal*, our students are making the most of these opportunities and accomplishing truly inspiring work. The scholarship that appears each year in this publication represents research excellence and creative endeavors of the highest order. UCR is at the forefront of discovery, with a world-class faculty, including two Nobel laureates, and state-of-the-art instructional and testing laboratories. UCR is creating a home for students, faculty, and community members to collaborate, test, and learn.

At UCR we are stewards of transformation; it is in our DNA to ensure that we create an environment and structure that fosters innovation to solve our community and world's greatest challenges. I am impressed by the dedication and innovation displayed by the students and their mentors in pushing forward in research despite the challenges faced by the pandemic.

I congratulate all the students who contributed to this edition of the Journal, and I express my sincere gratitude to the faculty mentors and staff members that supported these students in their scholarly endeavors.

Sincerely,

Kim A. Wilcox
Chancellor

FROM THE VICE PROVOST AND DEAN, UNDERGRADUATE EDUCATION

Engaging with research is about discovery in two important ways. First, in the university context, students are often encouraged to engage with research as they explore and discover their specific areas of scholarly interest. A student's area of research interest may be sparked within the context of a class, learning about a professor's research area through a lecture or during office hours, or by exploring the published literature on a topic. For others, discovery and engagement with research might be inspired by an issue, topic, or problem they would like to solve in the world or a community they care about and wish to serve.

Secondly, the theme of discovery through research is also about the researcher—engaging in inquiry, question-asking, data-generation, and thinking about ways the work might impact the audience or topic being explored. This was certainly the case for me. I was introduced to research about halfway through my undergraduate student experience. As a first-generation college student this was perhaps one of the most formative experiences of my college career. I explored the factors that contributed to high school student success, and this experience helped me discover my identity as an emerging researcher. Equally important to my identity as a researcher was my involvement in the honors program, my summer conducting research with a professor at another university, and my experiences as a McNair Scholar. I saw first-hand how engaging with research helps facilitate discovery in so many important ways.

To a large degree, this volume of published work in the *UCR Undergraduate Research Journal* reflects discovery by our students who have demonstrated a commitment to inquisitiveness, knowledge creation, and now, knowledge dissemination. The student-authors in this volume serve as a reminder that engaging with research is both an individual commitment to a goal but also an endeavor that is likely supported by a faculty mentor, a community, and a community of researchers that helps facilitate a student's success. Finally, this volume serves as an inspiration to researchers everywhere, some of whom may have inspired the work represented in this volume, and those future student-researchers who may be at the beginning stages of their own research journeys.

Congratulations to all of the contributors in this important volume!

Louie F. Rodriguez
Vice Provost and Dean, Division of Undergraduate Education
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It is with great pleasure that we present UC Riverside's 17th edition of the *Undergraduate Research Journal*. We have had the honor of collaborating with emerging minds across campus in developing this publication. It is the collective efforts of everyone involved and the time they provide that ensures the continuation of this journal with standards that reflect the excellence that UCR represents. Congratulations to the authors for your commitment—your courage to put your ideas out for criticism and refinement in an environment unfamiliar to many of us. Doing so is commendable and serves as a testament to the community of scholars you have worked with. Your achievements, found in this 17th edition, will forever be a part of the Journal's legacy. Congratulations are also due to the Student Editorial Board and Faculty Advisory Board–your diligence and dedication to the publication process have ensured the quality and success of the Journal. We are remarkably grateful to have been part of the outstanding team that made this edition possible.

Sincerely, Mahnur Bharucha and Andrew Martin, Co-EIC's

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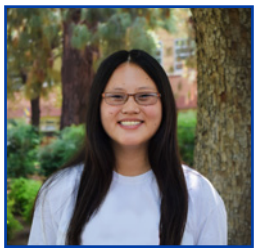

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FROM THE FACULTY ADVISOR BOARD

Since *UCR's Undergraduate Research Journal* started 17 years ago, it has published over 165 scholarly articles across many fields. These papers represent the commitment of our undergraduate students to performing independent research as part of their undergraduate experience. Because undergraduate research can often form part of a larger work with many contributors, the importance of the student's contribution can sometimes be lost. With the *Undergraduate Research Journal*, students can publish their work as first authors before the end of the academic year through a peer-review publication process. The paper becomes a part of students' professional experience, contributing to their record of scholarly achievement. The Journal's submission and review process is run by undergraduates who form the Student Editorial Board, working with members of the Faculty Advisory Board. We owe a debt of gratitude to these students for their professionalism and dedication to the review and preparation of the articles in this issue. We are also grateful for the participation of the members of the Faculty Advisory Board in guiding the reviewers. I would like to thank Gladis Herrera-Berkowitz for her work in supporting and guiding the process every year, Lisa Des Jardins for providing Graphic Design assistance, Jennifer Kavetsky for her support of our authors, as well as Undergraduate Education for their funding support. If you are interested in publishing your undergraduate research at UCR, consider submitting to our next issue!

Prof. Morris F. Maduro
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ABOUT THE COVER

Victoria Van Hulle

Victoria Van Hulle is majoring in Neuroscience and will be a Junior in Fall 2023. She graduated from Riverside STEM Academy High School in 2021. In her junior year, she plans to get involved in the incredible research opportunities that UCR has to offer. Her love for photography started at a very young age and has blossomed over time. She enjoys taking photos at the UCR Botanical Gardens in her free time.

Hummingbirds are tiny magnificent flying birds symbolizing joy, beauty, spirits, and angels. They are an inspiring sign of hope and good luck. I had the opportunity one Spring day at the University of California, Riverside Botanical Gardens to capture a hummingbird in flight. This beautiful hummingbird had a green and blue jewel-like body along with iridescent wings and was delightful to watch. I took many pictures of the hummingbird I named Annie hovering over a red flower from the Chuparosa plant. I got a beautiful view of Annies' iridescent pearl-like wings as the sun reflected through them.

Validation of a Virtual Reality Flanker Task

Radhika Amin, *Department of Molecular, Cell, and Systems Biology*
Phoebe Tat, *Department of Psychology*
Aaron Seitz, *Ph.D., Department of Psychology*

ABSTRACT

The Flanker task is a neuropsychological test designed to measure inhibitory control–the ability to ignore automatic responses in order to reach higher goals. The traditional, tablet form of the Flanker task is widely used and well-cited. Recently, the UCR Brain Game Center has developed a Virtual Reality (VR) Flanker game. After the project is created, the next step is to validate its usefulness with a pilot study, which is the focus of this research paper. This pilot study addressed two central research questions. The first research question was: would the newly developed VR Flanker task measure inhibitory control as well as the traditional, tablet Flanker task? In Pilot Study 1, participants were first randomly assigned to either the VR or computer form of the Flanker task, and then utilized the alternative platform in the second session. The second research question was: would different versions of the VR Flanker task yield different measurements of inhibitory control? In Pilot Study 2, participants were randomly assigned to either the Regular VR Flanker task or the Jitter VR Flanker task during the first session. The second session employed the version that participants were not assigned to in the previous session. The study compared the standard performance measure of the difference in reaction time between congruent and incongruent tasks (Flanker effect) in both Pilot studies. The results demonstrate that VR Flanker tasks are a valid way to measure inhibitory control. Pilot 1 found that Flanker effects produced in the tablet and VR Flanker tasks are similar, while Pilot 2 found that greater Flanker effects could be produced with a different presentation of stimuli in the virtual environment. Once it is established that the VR task measures inhibitory control as well as the tablet version, there would be multiple benefits for neurological tools and testing in different populations. The advantage of a VR Flanker game is that it can serve both as a fun method to assess Flanker effects and as a training tool. Long-term use of a validated VR Flanker task could allow us to emulate real-world situations and lead us to better understand populations struggling with inhibitory control.

KEYWORDS: Flanker, virtual reality, inhibitory control

FACULTY MENTOR - Dr. Aaron Seitz, Department of Psychology

Professor Aaron Seitz is a well-established investigator and internationally recognized as an expert on mechanisms of perception attention and learning and memory using behavioral, computational and neuroscientific methodologies. As the Director the UCR's Brain Game Center for Mental Fitness and Well-being, he is committed to translating lab-based research into approaches to benefit life in the real world. The lab works to disseminate game software instrumented with expert knowledge to optimize human brain processes with an aim to make scientifically principled brain games that translate to performance in real-life activities. His research led to new insights regarding the roles of reinforcement, attention, multisensory interactions, and different brain systems in learning.

Radhika Amin

Radhika is a fourth-year Neuroscience major. She has worked as a Research Assistant and Assistant Research Coordinator at the UCR Brain Game Center for the past 4 years. With funding from the RISE Internship and the Campbell Fellowship, she has had the opportunity to study the interaction of brain structure and function in diverse populations. Radhika is currently President of UCR Best Buddies and Student Facilitator of the R'Course Cognitive Assessments and Their Applications. She hopes to pursue further education and conduct health-related research, finding novel ways to help individuals that are affected by cognitive disorders.

Validation of a Virtual Reality Flanker Task

INTRODUCTION

The Flanker Task

The Flanker task is a neuropsychological task in which the participant must respond to a centrally presented target stimulus surrounded by distractor stimuli (**Figures 1 & 2**) (Davelaar, 2009). The Flanker task measures inhibitory control, a crucial executive function that is heavily relied upon during daily activities. Inhibitory control is the ability of one to control their behavior and ignore external lures to reach higher goals (Diamond, 2013). For example, a student who chooses to ignore a text message during a lecture to pay attention to the professor is exhibiting inhibitory control because they are ignoring external lures and focusing on the larger goal of doing well in the class. Inhibitory control is an important facet in the domain of cognitive function, as it influences daily decision-making. The Flanker task—the difference in reaction time between congruent and incongruent trials—is a primary measure of inhibitory control in the Flanker task. In congruent trials, the target stimulus is in the same orientation as the distractor stimuli, while in incongruent trials, the target stimulus is in a different orientation than the distractor stimulus (Davelaar, 2009). The traditional, tablet version of the Flanker task is the gold standard in the field of neuropsychology to measure inhibitory control. The UCR Brain Game Center has recently developed a VR version of the Flanker task, and the properties of this task are examined in the present study.

Research Aims

This paper summarizes the results from our Pilot studies, which aimed to validate if VR was a viable way to deliver Flanker stimuli and measure inhibitory control. Because these studies are expensive and time-consuming, pilot studies are necessary to establish proof of concept. Small-scale studies also help identify potential issues when the project is scaled. In these preliminary Pilot studies, we administered the different Flanker tasks to only a small group of individuals with the intent of later moving to larger populations to then fully validate the VR Flanker task. Hence, our first research question was: would our newly developed VR Flanker task measure inhibitory control as well as the traditional, tablet Flanker task? Once it was established in Pilot 1 that the VR Flanker task successfully measured inhibitory control, we developed and studied different versions of the VR Flanker task in Pilot 2. Our second research question was: would different versions of the VR Flanker task yield different measurements of inhibitory control? To answer both research questions, we compared and analyzed the Flanker effect (difference in reaction times between congruent and incongruent trials) averages of the participants across the different types and versions of Flanker tasks.

Previous Research

There are several validated versions of computerized tablet Flanker tasks (Davelaar & Stevens, 2009; Grainne, 2009; Kramer et al., 2013; McLoughlin et al., 2009; Oeri et al., 2019; Shimada et al., 2015). For instance, the UCR Brain Game Center has previously validated a computerized tablet version of the Flanker task, which is administered in several batteries to measure inhibitory control in different populations. In contrast to a tablet task, a virtual reality (VR) task could provide additional control in designing environments that could not be replicated in real-life experiments (Morel et al., 2015). After validation of the VR Flanker task, it could be utilized to study the inhibitory control abilities of populations that struggle with inhibitory control. Manipulating real-world situations in VR Flanker environments may also offer a therapeutic benefit to individuals of such populations in the future. These situations that were once unable to be studied can now be safely replicated in a lab setting. A VR Flanker task could furthermore serve as an enjoyable method to assess Flanker effects and as a training tool. It is important that participants are engaged and exert effort while performing

the Flanker task because it allows for a more accurate measurement of their inhibitory control abilities. While a few VR Flanker tasks have been validated, they are not easily accessible (inexpensive and able to be administered in a variety of lab contexts) and do not require the participant to use their full range of arm motion (Armstrong et al., 2012; Ribeiro et al., 2021). For instance, VR Flanker tasks in previous studies have only required participants to use finger taps or hand movements to respond to stimuli (Ribeiro et al., 2021).

Current Research

The Brain Game Center's recently created VR Flanker tasks address several of the aforementioned issues. Our VR Flanker task uses full body movement and could become accessible for individuals to use. We first developed Regular VR Flanker, which was a replication of the Tablet Flanker task on a virtual platform. Regular VR Flanker consisted of the stimuli spawning or originating at the same place in the virtual environment. In Pilot 1, we compared Flanker effects on our developed VR Flanker with the Brain Game Center's tablet form of the task. Then, modifications (i.e., changes in timing and placement of stimuli) were made on the VR Flanker task. Jitter VR Flanker consisted of stimuli that were originating at different places in the virtual environment. In Pilot 2, we Flanker effects of our Regular VR Flanker were compared with our Jitter VR Flanker.

MATERIALS AND METHODS

Participants

The study was divided into two pilot studies within a three-week period spanning early to mid-April 2023, with 9 students (66.7% female, 33.3% male) participating in Pilot 1 and 7 students (57.1% female, 42.8% male) participating in Pilot 2. The distribution of participants' ages ranged between 18-30 years old. Due to the current Pilot studies serving as a preliminary experiment to determine its reliability and feasibility of deployment upon a larger population, the small total sample size reported does not reflect the full scale of our research. Each pilot was further partitioned into two sessions to counterbalance the tasks and eliminate order effects, with the second session scheduled between 1 to 5 days after the first session. Participants were randomly selected using UCR Brain Game Center's roster of undergraduate research assistants and were not provided compensation for their participation. Each selected participant was asked to volunteer their time for a short activity and did not receive further details about the study. All participants indicated having either normal or corrected-to-normal vision using supplemental eyewear (e.g., glasses, contacts). Participants from both pilots were administered a written consent form before Session 1 and a feedback survey after Session 1. All tasks within the current study were administered in a quiet, enclosed room with minimal decor to reduce potential distractions and interference with participant performance.

Equipment Specifications

The Tablet Flanker task was conducted from the UCR Brain Game Center application, Recollect, via a stock 32 GB memory 6th-generation iPad running iOS 15.4. The VR Flanker task, developed by the UCR Brain Game Center, was administered using Meta Platforms' second-generation virtual reality headset, a stock 128 GB memory Oculus Quest 2 running v38.0. In Pilot 1, researchers randomly assigned participants to begin Session 1 with the tablet Flanker task or the VR Flanker task. In Pilot 2, participants were randomly assigned to begin with the Regular VR Flanker or the Jitter VR Flanker. To eliminate order effects, participants utilized the device they were not assigned to in Session 1.

The UCR Brain Game Center developed both the Tablet and VR versions of the Flanker task using a small selection of programs installed on a Windows 10 operating system. The Tablet Flanker task was developed using Unity, a game

engine supporting desktop and mobile platforms, while the VR Flanker task was developed using Unreal Engine 4, a game engine designed for desktop applications. The assets within the VR Flanker task, such as the glowing sabers and interactive targets, were modeled in Blender, an application supporting the modeling, texturing, and rendering of 3D objects.

Materials

UCR Brain Game Center's tablet Flanker task instructs participants to quickly indicate if the centrally presented arrow amongst a set of five arrows is pointing to the left or the right by pressing the corresponding triangular button (**Figure 1**). For instance, in Figure 1, the participant would respond with "left" since that is the direction of the middle arrow. Researchers did not provide further information beyond the task instructions.

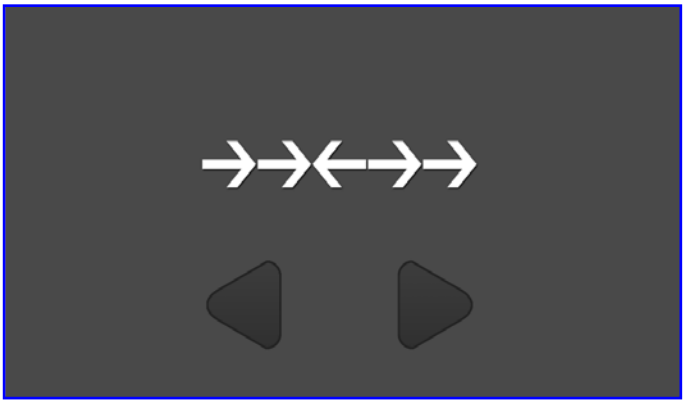

Figure 1. Tablet Flanker.

In our VR Flanker, participants are spawned into an immersive, three-dimensional environment wielding two glowing sabers. They are presented with a series of targets containing visible arrows and must slash through them by grasping a handheld controller and moving their arm along the horizontal axis depending on the orientation of the centrally presented arrow (**Figure 2**). To ensure accuracy, researchers instructed participants to capture their full range of motion by moving their arms and shoulders rather than limiting the movement to their wrists. The player's score, which reflects the number of correct responses provided within the time limit of each trial, is displayed above the targets and can be monitored throughout the session.

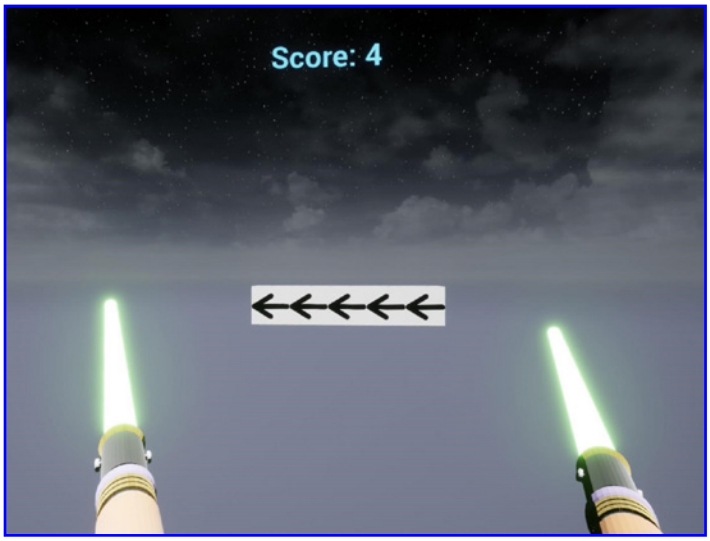

Figure 2. VR Flanker with no modifications made (i.e., Regular VR Flanker).

The Jitter VR Flanker variation used in Pilot 2 follows the same rules and objectives as the Regular VR Flanker, with the exception that targets appear in randomized areas rather than consistently spawning in the center of the screen/display (**Figure 3**).

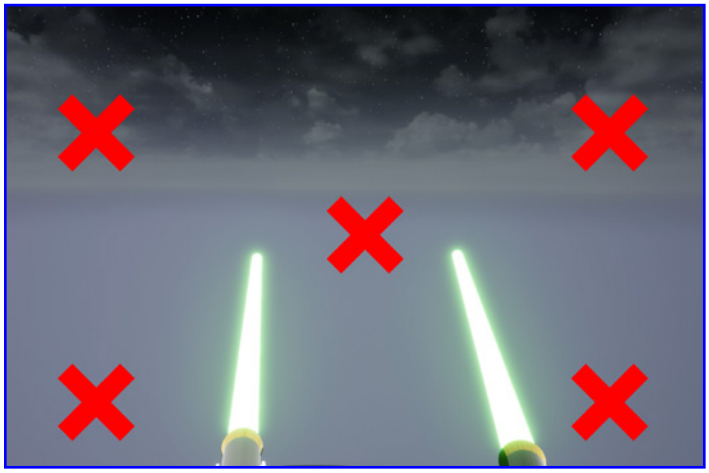

Figure 3. Possible spawn points for Jitter VR Flanker. Red Xs indicate possible spawn points.

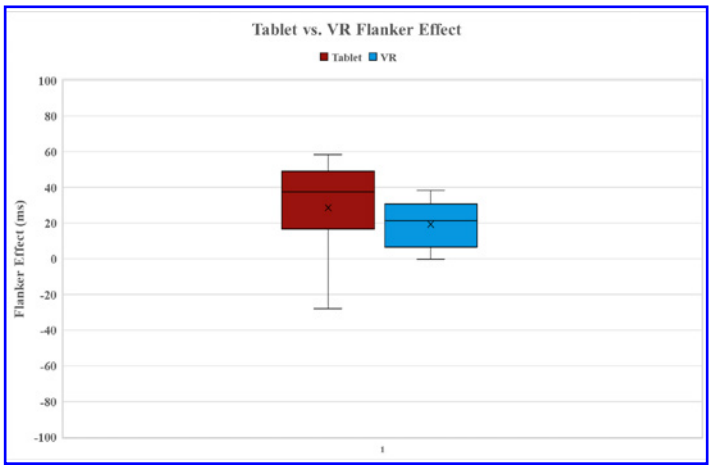

Figure 4. A box plot illustrating the Flanker effect across Tablet and VR Flanker.

RESULTS

Participants maintained similar performance across both sessions in Pilot 1, with the VR Flanker task averaging 19.25 milliseconds (ms) with a range of -27.96ms to 38.26ms and a standard error of the mean (σ_M) of 10.71ms, compared to the tablet Flanker task, which generated an average of 28.59ms and ranged -0.26ms to 58.52ms (σ_M = 4.51ms) (**Figure 4**).

Data from Pilot 1 demonstrated that we were able to achieve similar Flanker effects in the VR Flanker sessions and the tablet Flanker sessions with a correlation coefficient (r) of -0.5424 and the T-test score (*t*) of 0.3201. As shown in **Figure 5**, tablet Flanker reaction times with correct responses for incongruent trials averaged to 819.56ms (σ_M = 29.33ms) and congruent trials averaged to 791.52ms (σ_M = 791.52ms), whereas the VR Flanker average reaction time for incongruent trials was 267.17ms (σ_M = 30.79ms) and 247.91ms (σ_M = 28.65ms) for congruent trials.

Pilot 2 compared the Flanker effects of our Regular VR Flanker with our Jitter VR Flanker results. We were able to achieve a Flanker effect in both VR versions of the task, with the Jitter VR Flanker effect yielding higher Flanker effects (*r* = -0.1527, *t* = 0.1593). The Regular VR Flanker produced an average Flanker effect of 36.98ms, with a range of -8.67ms to 66.70ms (σ_M = 4.33ms), and the modified Jitter VR Flanker held a comparable average of 48.83ms, with a range of 36.90ms to 65.40ms (σ_M = 9.35ms) (**Figure 6**).

On average, the reaction time of correct responses for incongruent trials in Regular VR Flanker was 473.34ms (σ_M = 25.97ms) and that of congruent trials was 436.36ms (σ_M = 21.96ms). The Jitter VR Flanker's reaction time for incongruent trials averaged 531.56ms (σ_M = 16.94ms) and averaged 482.73ms (σ_M = 13.55ms) for congruent trials (**Figure 7**).

Consistent across both pilot studies, participants completed a feedback survey about their experience with the assessments.

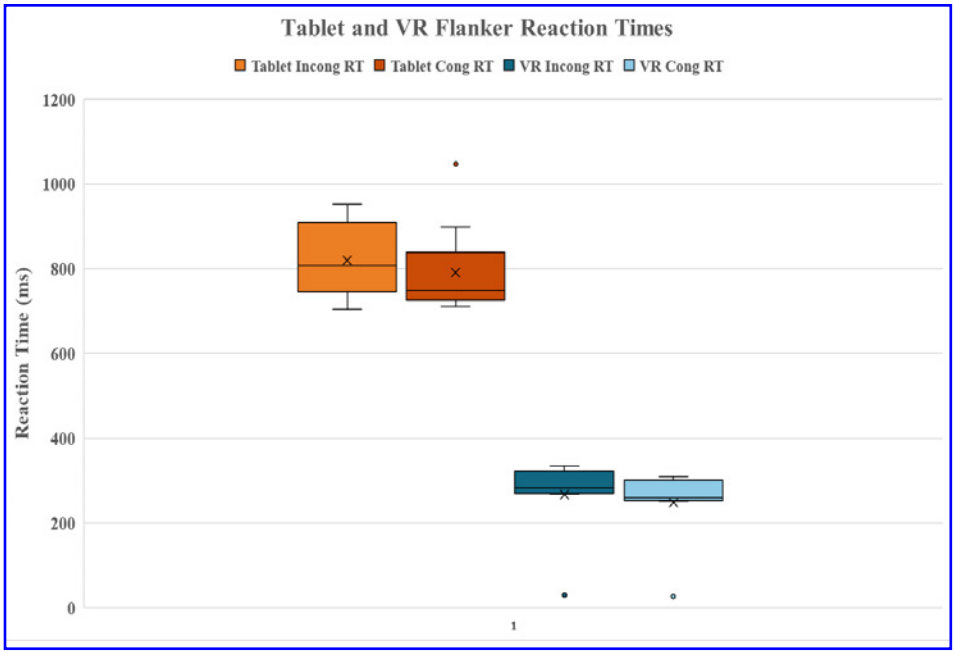

Figure 5. The average response times to stimuli between Tablet and VR Flanker, with considerations of congruency in the targets

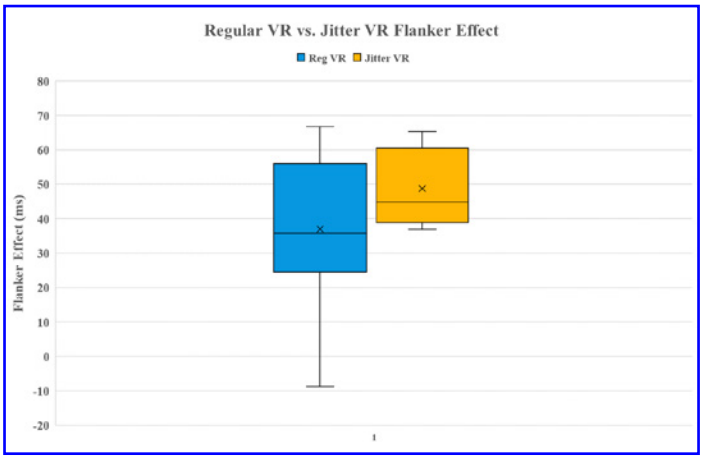

Figure 6. A box plot demonstrating the Flanker effect between the Regular VR Flanker task and the Jitter VR Flanker task.

In Pilot 1, when asked to choose the most challenging evaluation between the tablet Flanker and the VR Flanker, a greater part of the participants reported that the tablet Flanker was more difficult than the VR Flanker (**Figure 8a**). Correspondingly, a greater number of participants also reported enjoying the VR Flanker task as opposed to the tablet Flanker task (**Figure 8b**). However, the measure of enjoyment levels was similar between the two tasks (t = 0.005353).

In Pilot 2, however, most participants reported encountering the most difficulty during the Regular VR Flanker assessment (**Figure 9a**). Despite these discrepancies in difficulty, participants noted similar enjoyment levels among each task (t = 0.2891) (**Figure 9b**).

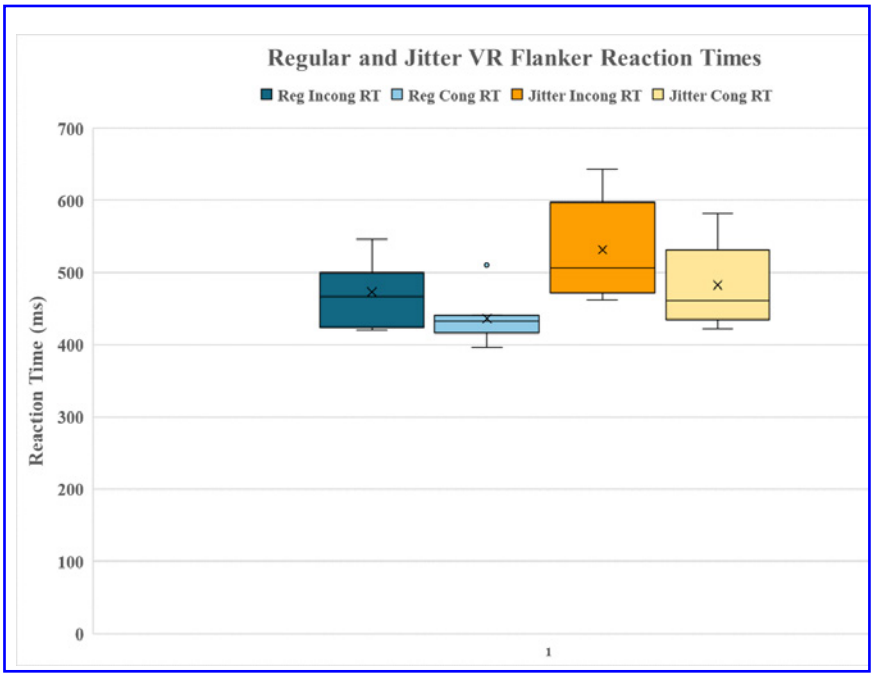

Figure 7. The average response times to stimuli between VR Flanker variations, with considerations of congruency in the targets.

DISCUSSION

In both Pilot studies, we aimed to validate that our VR Flanker task is a viable option for measuring inhibitory control while also studying the properties of different versions of the VR task. Pilot 1 aimed to recreate the Flanker effect of the tablet version of the task within the context of VR. The sessions in Pilot 1 used different forms of stimuli, yet the data extracted from the VR Flanker sessions (shown in **Figure 9**) closely resemble the results derived from the VR Flanker sessions. Therefore, we can deduce that our VR context successfully created a Flanker effect and the two tasks measure the same construct of inhibitory control. Overall, the data gained from the study demonstrates that the Flanker effect was produced from both of our developed VR Flanker tasks. The objective of Pilot 2 was to create a Flanker effect in the VR versions of the Flanker task, while also building upon the foundation created by Pilot 1 and further identifying which forms of stimuli would elicit a greater Flanker effect. A larger Flanker effect shows that participants likely experienced more conflict, causing them to exert greater inhibition when switching between congruent and incongruent trials. This allows us to better gain an

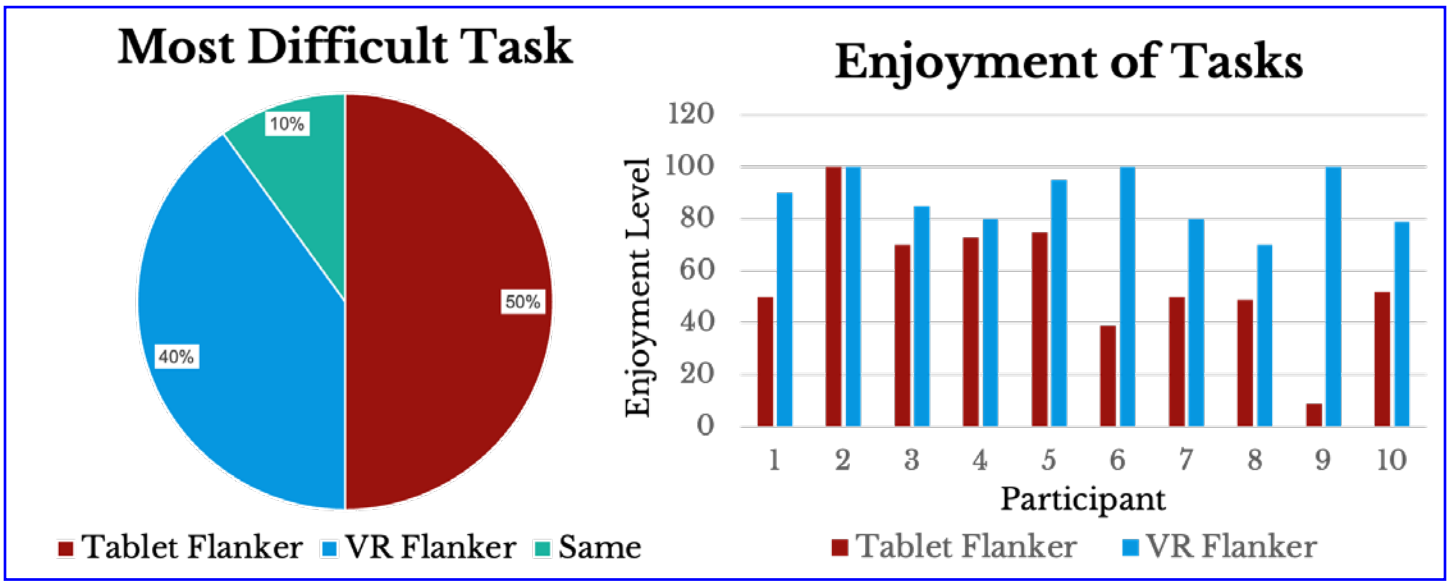

Figure 8(a,b). A visualization of the survey responses we received following the first session of Pilot 1. **(a)**. When asked to report which of the two tasks they found most challenging, the participants' responses. **(b)**. Distribution of individual submissions reporting enjoyment levels for each task.

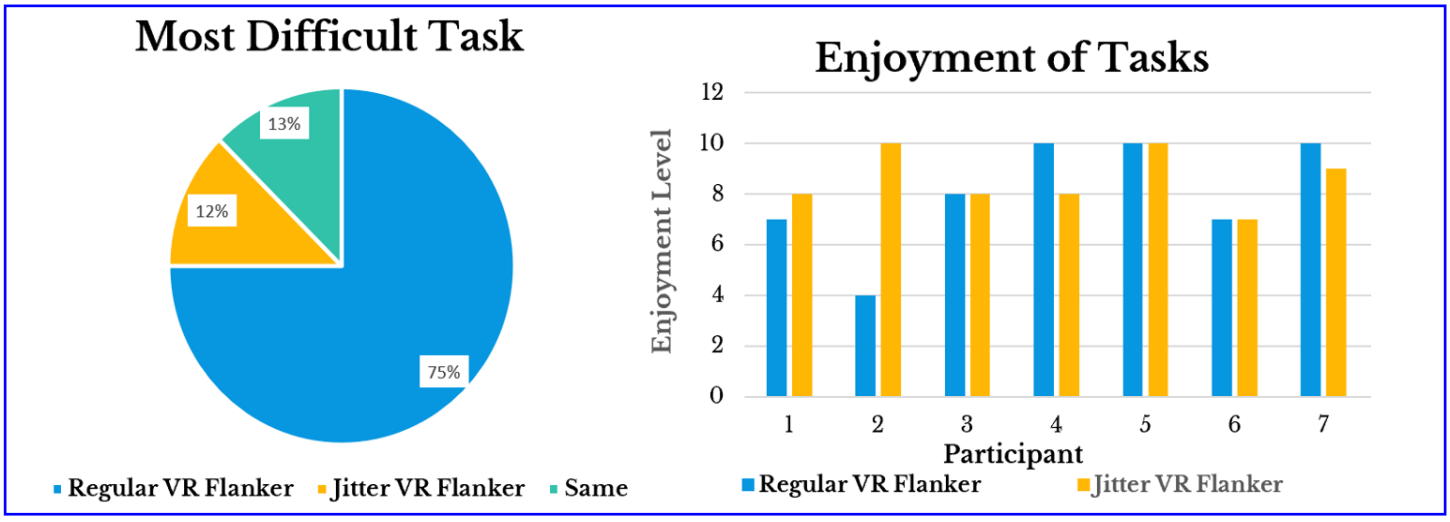

Figure 9(a,b). A visualization of the survey responses we received following the first session of Pilot 2. **(a)**. The participants' responses when asked to report which of the two variations of VR Flanker they found most challenging. **(b)**. Distribution of individual submissions reporting enjoyment levels for each variation.

accurate measurement of the participant's true inhibitory control abilities when they need to exercise inhibition in life situations. Results from both Pilot studies indicate that we achieved a Flanker effect in our VR Flanker tasks. Hence, this supports that our VR Flanker task can consistently and accurately measure the inhibitory control abilities of individuals. We can infer that the Jitter VR Flanker variation successfully produced a greater Flanker effect through its spatial experimentation. The placement of stimuli in different areas in the virtual environment may require greater

cognitive processing of the frontal lobe, leading to a greater Flanker effect. In Pilot 1, participants found the difficulty of the tablet and VR versions were similar. However, a greater number of participants found the VR version more enjoyable. This suggests that the VR environment may yield higher engagement levels from the participants, while also maintaining the same level of challenge. In Pilot 2, participants found the Regular VR task to be more complex than the Jitter VR task, but the enjoyment of the tasks was similar. This was unexpected, as we predicted the participants would have greater difficulty responding to the dispersed stimuli in the Jitter VR task than in the Regular VR task. Our prediction, however, was supported by the larger Flanker effect produced in the Jitter VR Flanker. Hence, this could indicate that participants may have been more alert during the Jitter VR task, causing them to perceive the task as being less difficult. Furthermore, participants may enjoy VR tasks regardless of the difference in the presentation of the stimuli in the VR environment.

FUTURE DIRECTIONS

Because this field is still developing, our research can help establish an important baseline. Current studies conducted at the UCR Brain Game Center designed to measure inhibitory control across diverse populations can implement our VR Flanker Task. In the future, we will administer the Regular VR Flanker task to a larger sample and examine the replicability of the preliminary results. We also plan to incorporate participant feedback as we further develop and change the Flanker tasks in VR. Moreover, we are working on extending the framework to add other inhibitory control tasks such as stopping and rule-switching. We are working on adding game elements such as levels, distractors, and point systems to increase participants' enjoyment of the task. These developments would help us contribute to the Brain Game project by allowing us to study how new VR versions of the Flanker task could create more significant Flanker effects. In the long term, analyzing performance measures of the VR Flanker task will elucidate the IC abilities of various populations. We could thus understand the general population better and help individuals that struggle with inhibitory control, such as those with substance use disorders or attention-deficit/hyperactivity disorder (ADHD).

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Food Justice is Health Justice: Benefits and Barriers to Connecting Student-Run Free Clinics with Student-Run Organic Gardens

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ABSTRACT

Food deserts in low-income and underserved communities pose a significant challenge to accessing healthy, affordable food, leading to poor health outcomes for residents. This project aimed to address this issue by examining the benefits and barriers that arise from connecting a student-run community garden (SRCG) with student-run free health clinics (SRFCs) to provide low-income patients with access to sustainable food and nutritional education. Using a case study research model, the study focused on investigating the organizational structure and potential mismatches between the two kinds of student-run systems. Birnbaum's Higher Education Organization Framework (1992) was utilized to analyze how loosely and tightly coupled organizations can affect the success of collaboration. Vignettes were developed based on participants' observational experiences, and lessons were derived from fields and discussions with participants. The study identified several organizational barriers that complicated the relationships between student-run organic gardens and student-run free clinics. The SRCG was a loosely coupled organization with little centralized cybernetic control, and the lack of organizational structure in SRCGs made it challenging to use SRFCs as outlets for providing fresh food to patients in the free clinics. Although connecting SRCGs and SRFCs shows promise for increasing food access and promoting health, the study found that it remains challenging to use gardens as food production systems without creating a more cybernetically controlled structure in the SRCGs. By analyzing the benefits and barriers that arise from connecting these two organizations, this project sheds light on the potential of student-led initiatives to address health disparities and promote sustainable food systems in underserved communities.

KEYWORDS: Food Insecurity, Community Garden, Free Clinics, Student Run, Cybernetic, Anarchic

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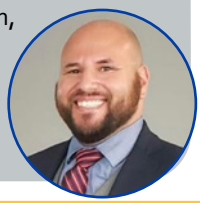

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Hana Baig is a fourth-year Biology major who currently studies how student-run community gardens and student-run free health clinics can work together to provide low-income patients with sustainable access to food and nutritional education. Funding for this project is provided by the UCR Chancellor's Research Fellowship. Currently, she is the president of Gardening Club, Vice-Chair of the Student Health Advisory Council, and a Dr. Eugene A. Moynier Scholar. After graduating, she hopes to pursue medicine with an emphasis in Public Health.

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INTRODUCTION

This project focuses on the integration of a student-run free health clinic with a student-run community garden, aiming to provide low-income residents with access to fresh produce and nutritional education. The partnership between student-run organic gardens and student-run free clinics can help ensure that low-income communities have the knowledge to make use of these resources. However, connecting these organizations can be challenging due to their different structures. This study examines the University of California, Riverside's R'Garden, and the Inland Empire Street Medicine (IESM) free clinic, using Robert Birnbaum's organizational framework theory to classify them as anarchic and cybernetic organizations, respectively. The R'Garden provides a space for growing fresh produce and is characterized as anarchic, while IESM aims to increase access to healthcare and is highly cybernetically controlled. The study explores the potential benefits and difficulties of connecting these two initiatives with different organizational structures.

The Inland Empire Street Medicine Free Clinic and the R'Garden began their partnership to address food insecurity in the Inland Empire region of California, specifically in Riverside. Food insecurity in Riverside is a prevalent issue affecting many individuals and families. According to a data dashboard from the Riverside County Community Health Assessment, which provides information on food insecurity rates and associated health outcomes in Riverside County, the food insecurity rate in Riverside County is 16.3%, which translates to over 240,000 individuals. This rate is higher than the national average of 10.5%. The problem of food insecurity is particularly acute in low-income communities and neighborhoods which have fewer grocery stores in comparison to neighborhoods of wealthier non-minorities (Berg and Murdoch, 2008; Powell et al., 2006; Block et al., 2008; Larson et al., 2009; Deener, 2017). With limited access to affordable, nutritious food, many residents in these areas face challenges in accessing healthy food options due to factors such as a lack of transportation, limited grocery store options, and a lack of income to purchase healthy foods. These barriers make it difficult to maintain a healthy diet leading to poorer health outcomes (Chung and Myers, 1999; Freedman, 1991; Hendrickson et al., 2006). These areas of High Poverty/Low Access (HPLA) are plagued with chronic illnesses such as heart disease, diabetes, obesity, and hypertension; the prevention of which depends on a proper diet. This mismatch between food availability and dietary needs puts these communities in a vulnerable position. Progress towards remedying this injustice and reducing health disparities begins with creative partnerships, such as connecting community gardens with patient clinics.

THEORETICAL FRAMEWORK

Birnbaum's cybernetic theories offer a comprehensive framework for understanding the functioning and organization of complex systems, particularly within the context of higher education institutions. This theoretical perspective focuses on the interconnectedness, communication, and feedback loops that exist within systems, emphasizing their adaptive nature and ability to respond to changes in the environment.

At the core of Birnbaum's cybernetic theories are two fundamental concepts: tight coupling and loose coupling. Tight coupling refers to strong interdependence and close communication between components of a system, leading to a higher degree of coordination and control. In contrast, loose coupling represents a more flexible and autonomous relationship between components, allowing for greater adaptability and resilience in the face of change.

Birnbaum also contrasts cybernetic and anarchic organizations, which represent two distinct approaches to organizing and managing complex systems. Cybernetic organizations emphasize tight coupling, strong interdependence, and close communication between

components, resulting in a higher degree of coordination and control. They often involve hierarchical structures and clear communication channels, allowing for efficient decision-making and resource allocation.

In contrast, anarchic organizations are characterized by loose coupling, where components have more autonomy and flexibility, leading to a greater capacity for adaptation and resilience. Anarchic organizations typically lack a formal hierarchy, and decisions are made through a collective process that encourages participation and diversity of perspectives. While cybernetic organizations are well-suited for stable environments where predictability and control are paramount, anarchic organizations tend to thrive in more dynamic and uncertain contexts, where adaptability and responsiveness are crucial for success.

The R'Garden can be classified as an anarchic organization based on its structure and characteristics. Anarchic organizations are characterized by a lack of formal hierarchy or authority, with power and decision-making distributed among all members. The R'Garden fits this description because it is an open space for students, faculty, staff, and community members to grow fresh produce, with no central authority or hierarchy controlling the organization. The R'Garden operates with a shared decision-making process and consensus-based decision-making structure, with all members having equal input and authority in the organization's decisions. The organization relies on a self-organizing system that allows members to take on tasks and roles based on their skills and interests, without a central authority controlling or assigning tasks. Furthermore, the R'Garden operates as an open community space, with no formal membership or registration process, and no official leaders[1]. This creates an inclusive environment where all members feel valued and empowered to participate in the organization's decision-making process.

The Inland Empire Street Medicine Free Clinic can be classified as a cybernetic organization based on its structure and characteristics. IESM's highly cybernetic structure is evident from its focus on providing healthcare services to low-income individuals experiencing homelessness in the Inland Empire. The organization relies on close collaboration between medical professionals, student volunteers, and medical students to provide comprehensive care to its patients. Additionally, IESM's hierarchical structure, with physicians and medical students serving as the primary decision-makers, further reinforces its cybernetic classification.

In the context of connecting student-run community gardens and student-run free clinics, Birnbaum's cybernetic framework provides a valuable lens for examining the interactions, communication, and adaptation within complex systems. By applying these concepts to the study of higher

1 While such forms exist on their website, R'interns do not have access to those forms and informally create new ones for limited audiences

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education institutions or other organizations, researchers can gain a deeper understanding of the underlying dynamics that drive decision-making, resource allocation, and overall performance. Using this framework, this study explores the potential benefits and difficulties of connecting student-run community gardens and student-run free clinics, despite differences in their organizational structures. Moreover, this framework can help identify areas for improvement and suggest strategies for enhancing adaptability, efficiency, and resilience within these systems.

STUDENT-RUN COMMUNITY GARDENS AND FREE CLINICS

Community and student-run gardens are an increasingly popular means of promoting healthy eating habits and providing fresh, locally-grown produce, as evidenced by studies such as Hume et al. (2022). Such gardens have the potential to improve access to fresh, nutritious food in low-income communities and provide numerous benefits to both gardeners and community members. In addition to promoting social interactions and networks, community gardens also have the potential to increase the cohesiveness of the community and improve overall well-being. Student-run gardens provide opportunities for hands-on learning experiences and leadership development for students. However, managing and sustaining these gardens can be challenging due to limited resources, administrative control, and seasonal variability, as noted by Jacobs and Kinzie (2012). It is important to consider organizational factors and sustainability to ensure long-term success. Despite these challenges, community and student-run gardens have been successful in promoting healthy eating habits, community engagement, and environmental sustainability, making them an important component of food systems and community development.

Student-run free clinics (SRFC) have become an essential part of healthcare provision for low-income and uninsured populations in the United States. As Holmqvist et al. (2012) highlights, SRFCs were founded in the early 1960s as a means of providing affordable community-based healthcare services. Typically, medical students staff these clinics under the supervision of licensed healthcare providers and operate on a voluntary basis. By providing free or low-cost healthcare services, SRFCs address the significant gap in healthcare access for underserved populations. Research indicates that SRFCs have a positive impact on health equity and improve health outcomes for low-income and uninsured populations. Birs et al. (2016) found that SRFCs reduce emergency room visits and hospitalizations among their patient populations. Additionally, SRFCs provide preventative care and early intervention for chronic conditions, leading to better patient outcomes (Holmqvist et al., 2012). However, the student-run nature of these clinics may create issues around supervision, liability, and regulatory compliance, which require careful management to ensure patient safety. This can lead to power imbalances, limited input from non-physician volunteers, difficulties working within short timelines, and potential limitations in decision-making processes.

Student-run free clinics (SRFC) and student-run community gardens (SRCG) have the potential to work together to address issues of food insecurity and healthcare access for low-income populations. SRCGs can provide fresh produce to SRFCs, improving the availability of nutritious food for patients, and creating a healthier community. Additionally, the social interactions and networks promoted

by SRCGs can lead to increased community engagement and a sense of well-being for SRFC patients. However, both organizations face limitations in terms of stability and sustainability. While SRCGs have an anarchic approach to organization, with little centralized cybernetic control, SRFCs have developed highly cybernetically controlled systems to ensure regulatory compliance and patient safety. These differences in organizational structure may create challenges in collaboration and communication between the two groups and may limit the input of non-physician volunteers in SRFC decision-making processes. Nevertheless, through effective collaboration and communication, these two organizations can work together to create a healthier and more sustainable community.

METHODOLOGY

This case study utilizes an exploratory design that relies on qualitative experiences throughout a 12-month (June 2022-June 2023) project period (Creswell, 2013; Creswell, 2014). Due to the ongoing nature of the partnership between the student-run free clinic (IESM) and the student-run community garden (R'Garden), this study only reflects key moments of particular events over the 12-month period. To analyze the organizational mismatches that occur when goals align, Birnbaum's Higher Education Organization Framework (1998) was used. This theoretical framework was used to analyze the implementation and organizational structures within the two organizations using qualitative methods such as content analysis and thematic analysis to identify themes and patterns that emerge from the data. The case study research model was used to examine organizational assets and challenges associated with connecting two kinds of student-run systems: student-run community gardens and student-run free clinics. Vignettes[2] were developed based on this author's observational experiences during the implementation of a partnership between these two organizations. This author was able to observe this partnership by initiating the collaboration through their leadership positions in the Inland Empire Street Medicine and Gardening Club, which is affiliated with R'Garden.

2 Vignettes are short stories that describe a specific moment in time

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FINDINGS

Theme 1: Harmony

Initiating and planning collaborations between organizations can be a complex and challenging process, particularly within student-led groups. Differences in organizational structures, goals, and cultures can create significant hurdles that must be addressed before any meaningful partnership can be formed. Such challenges may arise from competing priorities, lack of resources, and conflicting communication channels. Additionally, limited experience and capacity in managing collaborative projects may also be a barrier for student-led groups. Without proper planning and coordination, partnerships can easily fall apart due to misunderstandings, misaligned expectations, and lack of commitment. Therefore, it is crucial for organizations to establish clear objectives, allocate resources effectively, establish open communication channels, and develop a comprehensive project plan to ensure successful collaboration. Despite these challenges, successful collaborations can have a significant positive impact on the organizations involved and their communities. By building on each other's strengths and overcoming weaknesses, organizations can achieve more together and create sustainable solutions to complex problems. The power of synergy is a key factor in the success of any partnership and can be harnessed through effective communication, mutual respect, and shared goals. The partnership between Street Medicine and R'Garden is a powerful example of how collaboration can create meaningful change. Although the two organizations had different missions, they recognized the potential benefits of working together. In this vignette, we see how these two organizations with distinct missions came together to achieve a common purpose and make a positive impact in their community. By leveraging each other's strengths and resolving each other's weaknesses, they were able to achieve more together than they could have on their own. This experience explores the power of synergy and how it can be harnessed to build successful partnerships between organizations.

Vignette 1: Avocados

With the onset of summer, crops at the R'Garden had visibly begun to thrive. As a result, an opportunity for collaboration between the Inland Empire Street Clinic and the R'Garden arose. Inland Empire Street Medicine's partnership with R'Garden was a promising collaboration that aimed to bring together two organizations with distinct missions to achieve a common purpose. However, as with any partnership, the logistics of the collaboration needed to be carefully planned and coordinated to ensure its success. The Street Medicine board members volunteered to help with the avocado harvest, which was the first project the two organizations decided to work on together. To make the harvest a success, they had to coordinate with one of the R'Garden interns to plan the logistics for the harvest. This involved setting a date, organizing transportation, and ensuring that everyone knew what was expected of them. On the day of the harvest, the R'Garden team showed the Street Medicine

volunteers how to pick avocados and transport the harvested produce back to the clinic. The Street Medicine team worked closely with the R'Garden interns to learn the most efficient way to harvest the fruit. They were able to pick a significant amount of avocados and transport them back to the clinic's fridge. The avocados turned out to be a popular addition to the clinic's resources and became an added source of nutrition for the patients. Moreover, the R'Garden team was able to distribute its products more widely in the community.

The success of this collaboration demonstrates the power of synergy when two organizations with different structures and goals come together to achieve a common purpose. By harnessing each other's potential and resolving each other's drawbacks, Street Medicine and R'Garden were able to achieve more together than on their own. This example illustrates how proper planning, effective communication, and mutual respect can overcome the challenges of initiating and planning collaborations between organizations, particularly within student-led groups.

Theme 2: Communication

The success of any partnership hinges on effective communication and a reliable supply of resources. In this vignette, "Corn," it is revealed how an unexpected lock on a community garden prevented a group of volunteers from accessing the produce they had picked, resulting in a lost opportunity for the community clinic. The incident underscores the need for consistency and reliability in communication to ensure successful partnerships. Additionally, there was a lack of clarity regarding who should be contacted in case of any setbacks or unexpected situations during the event. The failure to communicate about the lock prevented the volunteers from accessing the produce, which highlights the importance of accountability in effective coordination.

Vignette 2: Corn

During the summer, the student leaders from Inland Empire Street Medicine and R'Garden were working together to plan and execute clinics. As part of their partnership, the Street Medicine team contacted the R'Garden interns to arrange for products to be picked and used at the clinic. After coordinating and setting a date to pick corn, the Street Medicine team arrived at the garden, picked the produce, and asked for permission to pick it up on the day of the clinic. The R'Garden interns agreed, and everyone confirmed the plan was in place. However, on the day of the clinic, the Street Medicine team found a lock preventing them from accessing the produce. They had not been informed of the lock and could not get in touch with the R'Garden intern who had the passcode. The lock had been added to prevent people from picking from each other's plots, which is a valid concern. However, putting a lock on the garden without informing those who need to access it prevents people from coming in. As a result, the Street Medicine team could not use the produce they had picked.

This experience highlights the importance of communication and accountability in successful partnerships. In this situation, we see that the collaboration between the two organizations hit a roadblock due to a lack of communication about the lock and failure to provide the passcode in a timely manner, resulting in wasted effort and resources. The Street Medicine team had volunteered their labor to pick the produce, but due to external factors beyond their control, they were unable to use it. Both parties need to be aware of the expectations and responsibilities of the other to ensure that they are working towards a common goal. Furthermore, the unreliable supply due to external factors such as the lock can lead to frustrations and difficulties in maintaining a successful partnership. Consistency and reliability in communication are essential to ensure that both parties can work together effectively toward their shared goal. Without proper communication and accountability, partnerships can easily fall apart due to misunderstandings and misaligned expectations. Therefore, it is crucial for organizations to establish clear objectives, allocate resources effectively, establish open

communication channels, and develop a comprehensive project plan to ensure successful collaboration.

Theme 3: Coordination

Coordinating activities and events in a student-run environment can be a complex and challenging process, particularly when different groups have conflicting schedules and priorities. In this vignette, we see how a hierarchical structure within a cybernetic organization (SRFCs) making a unilateral decision led to a mismatch in decision-making that affected the success of receiving produce from R'Garden. This situation highlights the importance of effective communication, collaboration, and consideration of different schedules and priorities to prevent similar mismatches in the future. It also sheds light on the difficulties of navigating various systems and structures within a student-run environment.

Vignette 3: Carrots

Two weeks prior to the scheduled clinic, the student leaders of the SRFC decided to reschedule the clinic to an alternative date. However, they were unaware that this decision would conflict with R'Garden's schedule. The R'Garden was unable to accommodate the clinic on that day due to undergraduate finals, causing a mismatch in scheduling. Despite the scheduling conflict, the SRFC proceeded with the rescheduled date, which fell on the weekend before undergraduate finals, creating an even greater scheduling conflict for undergraduate volunteers.

As many volunteers had planned beforehand to not attend the clinic due to upcoming finals, this resulted in fewer undergraduate volunteers for the SRFC. The medical students had to compensate for our absence, performing tasks that were typically the responsibility of undergraduate volunteers. Unfortunately, due to the short notice, communication with R'Garden was not possible. The R'Garden interns were also having finals, making it impossible to obtain fresh produce from them. As a result, the SRFC had to purchase produce from a grocery store and use a few leftover carrots.

This situation highlights the crucial role of effective communication, collaboration, and consideration of various schedules and priorities to prevent conflicts in the future. It emphasizes the importance of inclusive planning and decision-making that considers the needs and perspectives of all stakeholders for a successful outcome. Moreover, it underscores the potential negative impacts of a hierarchical decision-making structure in such organizations. This is particularly true when decisions are made unilaterally, without proper consideration for other groups within the system.

Theme 4: Evolution

The R'Garden has long operated under an anarchic structure, where decisions are made through a collective process with minimal hierarchy. However, this structure has led to gaps in leadership and funding issues, which have prevented the garden from expanding and improving its facilities. The absence of a clear hierarchy and decision-making process has led to difficulties in obtaining the necessary resources to expand and improve the garden's facilities. Without clear leadership, it can be challenging to identify funding opportunities and connect with key partners in the community. These gaps in funding and leadership limit R'Garden's ability to achieve its goals and promote sustainable agriculture and food justice in the community. Institutional structures that are cybernetic can offer valuable guidance and support for organizations operating within them, providing resources, funding, and connections to other stakeholders in the community. The R'Garden is an anarchic structure focused on promoting sustainable agriculture and food justice in their community. By utilizing the resources and support provided by more cybernetically structured groups such as the Center for Healthy Communities (CHC) and the Riverside University Health System (RUHS), the R'Garden can augment its capacity to identify funding opportunities and connect with key partners in their community. Additionally, partnering with the use of cybernetic organizational

structures allows the R'Garden to track their progress and effectiveness over time, and adjust their operations as needed to meet emerging challenges and evolving community needs.

This vignette highlights the importance of utilizing cybernetic organizational structures to support and enhance the work of community organizations. To address these issues, the R'Garden has recently introduced a referendum that seeks to provide consistent funding and redistribute responsibilities through new positions and committees. This move would enable the garden to become more tightly coupled and cybernetic, allowing for better organization and inter-organizational collaboration. In this vignette, we see how the referendum was initially met with challenges, persevered, and is paving the way for a more effective approach to addressing food insecurity.

Vignette 4: Radishes

Prior to the study, the R'Garden was advocating for a student referendum to address funding gaps that resulted in leadership issues. This advocacy lasted for a year and made significant progress. I learned more about the referendum during the project from a College of Natural and Agricultural Sciences (CNAS) senator. The senator believed in community gardens, especially in Riverside, where food insecurity is a concern. The referendum was nearly complete, but it faced barriers due to politics within the Associated Students of UCR (ASUCR). A graduate student who had been advocating for the referendum fell ill, which slowed its progress as there was no one else from the R'Garden working on the referendum.

The CNAS senator met with R'Garden representatives, past senators, ASUCR Pro Staff, and the CNAS Executive Committee to push the referendum. The entire committee, including the Dean of CNAS, unanimously supported the referendum. However, the Dean later sent a memo stating that they would only offer technical support and would not handle the R'Garden's finances, which was a significant obstacle. The R'Garden faced additional challenges, including a delay in formal voting in the referendum instructions due to the Judicial Branch's inability to reach a quorum. Nonetheless, the referendum was approved a week later, meeting two-thirds of the Senate's approval.

Introducing this referendum is a step forward for R'Garden's facilities and faculty in evolving a cybernetic structure. With consistent funding, they will be able to expand their resources for improving the garden's services, and administrative positions will be added to distribute responsibilities. A committee will also be formed to facilitate outreach, communication, and inter-organizational collaboration. Although the process of advocating for this referendum required a considerable amount of time, all parties involved expressed satisfaction that it can now proceed toward combating food insecurity. The R'Garden's commitment to evolving its structure and securing the resources it needs will bolster its mission to combat food insecurity in the community. This experience demonstrates how resilience, cooperation, and strategic planning can drive meaningful change within an organization and the broader community it serves.

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CONCLUSIONS

The findings of this project demonstrate the potential benefits of connecting student-run community gardens with student-run free health clinics to address food insecurity in low-income and underserved communities. Through the utilization of their respective strengths and addressing each other's weaknesses, the collaboration between R'Garden and Street Medicine demonstrates how these organizations can work together towards a common purpose of improving the health outcomes of underserved populations.

The paper highlights the importance of aligning structures, values, and governance to create a functional relationship between SRFCs and SRCGs, as well as recognizing and respecting each other's differences and working towards a shared goal. Effective communication is crucial to ensure successful collaboration.

Different values can also create tensions, but by recognizing and respecting each other's values, organizations can find common ground and work towards a mutually beneficial relationship. The study highlights gaps in leadership, communication, and accountability that can hinder the success of collaborations between organizations. However, the benefits of synergy demonstrated through the collaboration of Street Medicine and R'Garden show how two organizations with different structures and goals can achieve a common purpose by capitalizing on each other's strengths and compensating for each other's weaknesses.

One of the primary themes that emerged in the analysis was the gap in leadership and accountability. In many cases, the leaders of SRFCs and SRCGs may have different ideas about the goals and direction of their organizations, leading to misunderstandings and a lack of shared vision. This gap in leadership can also lead to a lack of accountability, as each organization may be focused on its own priorities rather than the success of the partnership.

Another theme that emerged was the importance of communication. Effective communication is essential to ensure that both parties can work together effectively toward their shared goal. Without proper communication, partnerships can easily fall apart due to misunderstandings and misaligned expectations. Therefore, it is crucial for organizations to establish clear objectives, allocate resources effectively, establish open communication channels, and develop a comprehensive project plan to ensure successful collaboration.

Despite these challenges, the analysis also identified several benefits of partnership between SRFCs and SRCGs, including the power of synergy. Collaboration can demonstrate the power of synergy when two organizations with different structures and goals come together to achieve a common purpose. Another benefit of collaboration is the opportunity for both organizations to learn from each other. SRCGs can teach SRFCs about sustainable food practices, while SRFCs can teach SRCGs about the importance of food security and access. By sharing knowledge and resources, both organizations can expand their impact and create positive change in their communities.

The study's limitations call for further research and data analysis to provide a more comprehensive understanding of the patient impact and the relationships between SRFCs and SRCGs. A quantitative analysis could provide a more comprehensive understanding of the patient impact and the relationship between the two organizations, by collecting data on various factors, such as the number of patients served, the frequency of visits, the types of services provided, and the overall health outcomes of patients who received care through the collaboration between the R'Garden and Street Medicine clinic. This data could be compared to data collected before the collaboration started, as well as data from patients who received care or services from either organization independently. Other quantitative measures could include analyzing the cost-effectiveness

of the collaboration, such as the amount of money saved by providing preventative care to patients who might have otherwise needed more expensive interventions. Surveys could also be conducted to collect data on patient satisfaction with the care they received, as well as their experiences with the collaboration between the two organizations.

The positive impact of this collaboration suggests that other student-run organizations can learn from this model and apply it to their own initiatives aimed at addressing community needs. This approach can also serve as a model for partnerships between grassroots organizations and cybernetic institutions, such as government agencies, to address complex societal issues. Ultimately, this project provides valuable insights into the potential of student-run organizations to positively impact their communities and the importance of collaboration in achieving their goals.

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All images used in this article were taken by Hana Baig.

Food Justice is Health Justice: Benefits and Barriers to Connecting Student-Run Free Clinics with Student-Run Organic Gardens

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Identification of Alternatively Spliced Genes in Metabolic Disease Pathways

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ABSTRACT

As of 2022, one-third of US adults experience metabolic diseases. Current therapies treat symptoms but do not address disruptions in signaling pathways of the liver that lead to the development of metabolic diseases. It is now recognized that many genes involved in metabolic disease pathways are alternatively spliced. This research aims to identify real alternative splicing events at genes that can serve as therapeutic targets. Alternative splicing is a critical process by which exons within pre-mRNA are either included or removed to generate diverse mRNAs and proteins. Transcriptomic data from the livers of both male and female mice under several different conditions–fed versus fasted, wildtype, and α7HMZ mice were analyzed for splicing events using an RNA-seq program, DEXSeq. α7HMZ mice express an alternative form of the transcription factor HNF4a, a critical liver and metabolism regulator. Current RNA-seq programs cannot distinguish alternative splicing from other activity occurring at the gene locus, so manual curation is necessary. Using a curation criterion, I manually analyzed 177 genes identified by the program for alternative splicing events. My analysis identified splicing events at mitochondrial genes usually expressed during fasting conditions and genes whose loss-of-function is implicated in obesity, hyperglycemia, and hypertension. Future research will analyze the mechanistic roles of these mitochondrial genes in various metabolic disease models.

KEYWORDS: alternative splicing, metabolic disease, RNA-seq, mitochondria, liver metabolism, obesity

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Shraddha Bhonsle

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INTRODUCTION

Millions of people across the world today suffer from debilitating metabolic diseases. According to the NIH, as of 2022, 1 in 3 US adults meet the criteria for metabolic syndrome (Metabolic Syndrome, NHLBI, NIH.), which includes a cluster of biological factors characterized by abdominal obesity, dyslipidemia, hypertension, and type 2 diabetes (Moore, 2017). With a predicted further increase in the incidence of metabolic syndrome, more people are at risk for developing more serious chronic conditions and comorbidities, making it urgent to understand metabolic disease pathways and identify improvements for current interventions. While current treatments and medications for these diseases have saved lives by treating symptoms, future therapies must address the metabolic dysregulation that occurs when tightly controlled signaling pathways in the liver go awry and lead to disease.

Recent studies have now recognized that much of the dysregulation involved in metabolic disease pathways result from alternatively spliced (AS) genes; thus, identifying these is critical to elucidating disease mechanisms (Baralle et al., 2017). We utilize the bioinformatics programs, DEXSeq and DESeq2, to analyze RNA-seq data from the livers of male and female mice under different conditions: fed versus fasted states; wildtype (WT) versus HNF4a7 HMZ. The 7HMZ mice express an isoform of the transcription factor HNF4a, a critical liver and metabolism regulator, that has been found to be upregulated in liver cancer, in the livers of mice fed high-fat diets, and in alcoholic livers (Fekry et al., 2018; Argemi et al, 2019). RNA-seq programs called 177 genes as loci of alternative splicing events. My research aims to manually verify the programs' identification of 177 alternative splicing events and identify those involved in metabolic disease pathways to screen for potential therapeutic targets for metabolic diseases.

Signaling Pathways and the Liver

Studying alternative splicing in the liver is important for understanding metabolic disease because the liver plays a critical role in regulating metabolism. The liver maintains metabolic homeostasis during fed and fasted states by employing tightly controlled signaling pathways (Kalra et al., 2017). During a prolonged fast, a drop in blood sugar decreases the need for insulin. A decrease in insulin increases glucagon and catecholamines, which activate AMP-activated protein kinase (AMPK), and in turn activates the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα) (Pawlak et al., 2013). As shown in **Figure 1**, PPARα subsequently increases the expression of genes involved in gluconeogenesis and glycogenolysis, processes to maintain glucose levels in the blood for vital organs such as the brain. As fasting continues, the liver also increases fatty acid oxidation, the process to produce ketone bodies for use as an alternative fuel source for the brain and other tissues (Cahill et al., 2006). If gluconeogenesis or ketogenesis is disrupted, it can lead to the onset of metabolic conditions such as hypoglycemia or ketoacidosis (Shimano, 2001).

In contrast, during the fed state, the liver shifts its focus to storing excess nutrients. Insulin levels increase, which promotes glucose uptake and glycogen synthesis in the liver. In individuals with insulin resistance, excess glucose and fatty acids accumulate in the liver, leading to the development of Non-Alcoholic Fatty Liver Disease (NAFLD), a condition characterized by fat accumulation in the liver. The liver also increases *de novo* lipogenesis to convert excess glucose into triglycerides, which are stored in adipose tissue or exported as very low-density lipoprotein (VLDL) particles (Cahill et al., 2006). This process is regulated by several transcription factors, including sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP) (Shimano, 2001). Dysregulation of these transcription factors (SREBP-1c and ChREBP) can contribute to the development of metabolic diseases (Shimano, 2001).

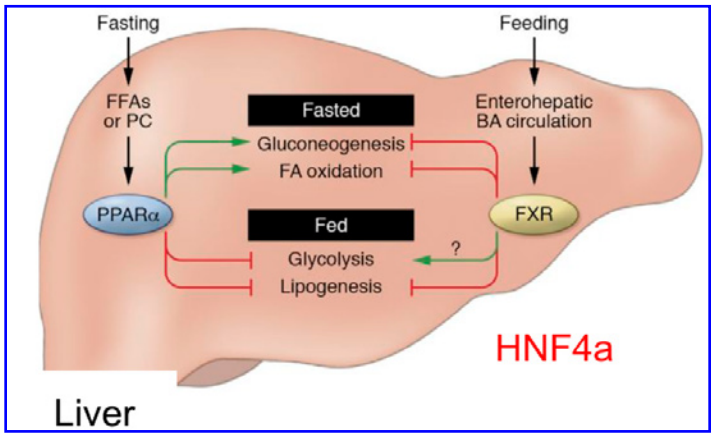

Figure 1. Liver's Pathway Mechanism of Action in Fed and Fasted states (modified from Preidis et al., 2017)

HNF4α and the Liver

Hepatocyte Nuclear Factor-α (HNF4α) is a highly conserved transcription factor. It is expressed in the liver and recognized as the master regulator of liver-specific genes as it is essential for the proper functioning of the adult and fetal liver (Battle et al., 2006.; Torres-Padilla et al., 2001). It is also expressed in other tissues including the kidney, pancreas, stomach, and intestine. The *HNF4A* gene consists of two promoters, P1 and P2, and along with alternative splicing processes, produces multiple variants or isoforms of the HNF4α protein (Ko et al., 2019). The two promoters are expressed under different conditions and play distinct roles in different tissues.

When the P1 promoter is expressed, the HNF4α1/α2 isoform of the protein is produced. When the P2 promoter is expressed, it gives rise to the HNF4α7/α8 (Chellappa et al., 2016) protein isoform. Under normal conditions, mice engineered to produce only P1-driven HNF4a proteins and mice producing only P2-driven HNF4a proteins appear healthy. However, when subjected to treatments that cause colitis or colon cancer, mice that produce only P2-HNF4a proteins experienced more colitis and developed more tumors than untreated mice due to increased expression of pro-inflammatory factors (Chellappa et al., 2016). This finding explains how the dysregulation of HNF4α in the liver can cause defective glucose homeostasis, dyslipidemia, and hepatic defects, many of which are observed in metabolic diseases (Hayhurst et al., 2001).

Alternative Splicing

Alternative splicing is a very common occurrence in the human genome. In fact, nearly 95% of genes with multiple exons undergo alternative splicing (Bhadra et al., 2020). This is the process by which 25,000 genes can produce 90,000+ proteins (E et al., 2013). As shown in **Figure 2**, introns in pre-mRNA are spliced out and the remaining exons in mRNA can be spliced together in different orders to produce different proteins. Since alternative splicing is critical to generating protein diversity, changes in splicing patterns have led to abnormal gene expression. This abnormal gene expression can later contribute to the pathogenesis of metabolic diseases such as diabetes, obesity, and non-alcoholic fatty liver disease (NAFLD) (Bhadra et al., 2020).

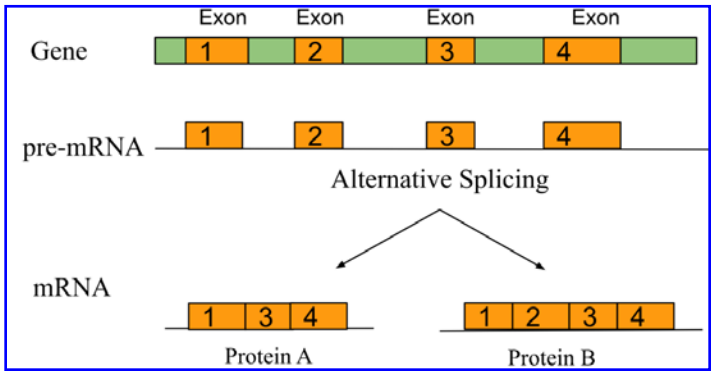

Figure 2. Alternative Splicing Mechanism. Alternative Splicing is a process by which exons within pre-mRNA are either included or removed to generate differentially spliced mRNAs. Splicing events generate diversity in protein products and can result in pathogenic isoforms that contribute to metabolic disease.

In the liver, alternative splicing is key to regulating the expression of HNF4α and other glucose and lipid metabolism genes. Specifically, studies have shown that changes in the splicing pattern of HNF4α can lead to dysregulation in the expression of genes involved in lipid metabolism, contributing to the development of NAFLD

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(Battle et al., 2006). Splicing of HNF4α can result in isoforms with different transactivation activities, suggesting a role in the regulation of liver-specific gene expression in metabolic disease pathways (Briançon et al., 2006).

METHODS

Young adult (16 to 20 weeks) male WT and HNF4α exon swap mice (α7HMZ) (Briançon and Weiss, 2006) (see Figure 3) were fed a standard lab chow (LabDiet, #5001, St. Louis, MO) and maintained in an SPF vivarium. Mice were fed or fasted for 12 hours from 10:30 PM to 10:30 AM (lights off at 7:30 PM, lights on at 7:30 AM) and euthanized by CO_2 asphyxiation at 10:30 AM followed by tissue harvest. Their livers were examined for changes in gene expression by RNAseq using DESeq2 software and Illumina sequencing (Deans et al., 2021). The RNAseq results were aligned to the mouse genome (mm39) and analyzed for alternative splicing using DexSeq software (Martinez-Lomelli, 2022). RNA-Seq #1 is from WT and a7HMZ exon swap mice in a mixed 129/Sv plus C57BL/6 background. RNA-Seq #2 is from WT and a7HMZ exon swap mice backcrossed into C57BL6/N. Liver samples from three mice per condition (triplicates) were prepared in an identical fashion. Both sets of data were analyzed in an identical fashion and compared for commonly splicing transcripts. Care and treatment of the animals were in strict accordance with guidelines from the Institutional Animal Care and Use Committee at the University of California, Riverside.

Statistical Modeling Software

DESeq2 uses a statistical model to accurately detect differences in gene expression by considering the relationship between the amount of gene expression and the variability in the data. The differential expression analysis involves comparing gene expression levels between two or more samples and identifying genes that are significantly upregulated or downregulated. DexSeq detects splicing events that are differentially used between two or more sample groups by modeling differences in read counts across exons of a gene and identifying exons that have significantly

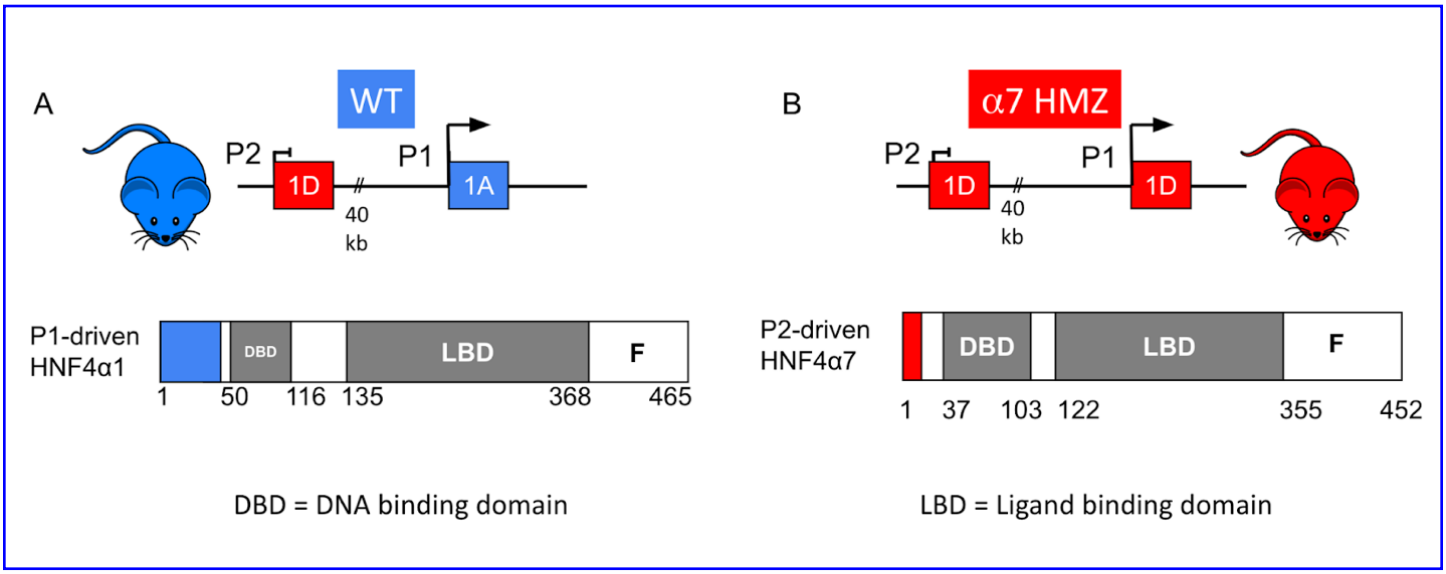

Figure 3. HNF4a exon swap mice. Schematic of mouse HNF4a gene in (A) WT and (B) a7HMZ mice showing the two promoters, P1 and P2, and the P1- and P2-driven HNF4a isoforms they express (bottom). DBD, DNA binding domain; LBD, ligand binding domain. Exon 1D and Exon 1A encode Activation Function (AF-1) and distinguish the two isoforms.

different usage between the samples being compared. This software was used to identify isoform-level changes in gene expression and understand how alternative splicing events may be associated with disease or other biological processes.

Exon-swap mice

HNF4 exon swap mice (α7HMZ) express an alternative form of HNF4A that is not expressed in normal adult liver (Braincon and Weiss, 2006). This isoform has been identified as a major isoform upregulated in liver cancer, in the livers of mice fed high-fat diets, and alcoholic livers making it a relevant model to study metabolic diseases (Fekry et al. 2018; Argemi et al, 2019). As shown in **Figure 3**, α7HMZ mice have the exon 1A coding sequence deleted at the P1 promoter and replaced by that of exon 1D using a plasmid construct so that they are homozygous for exon 1D and referred to as 'α7HMZ-only' mice (Chellappa at al., 2016). This minimal intervention makes it so the mice do not have obvious phenotypic defects or lethal defects as HNF4a was not completely knocked out.

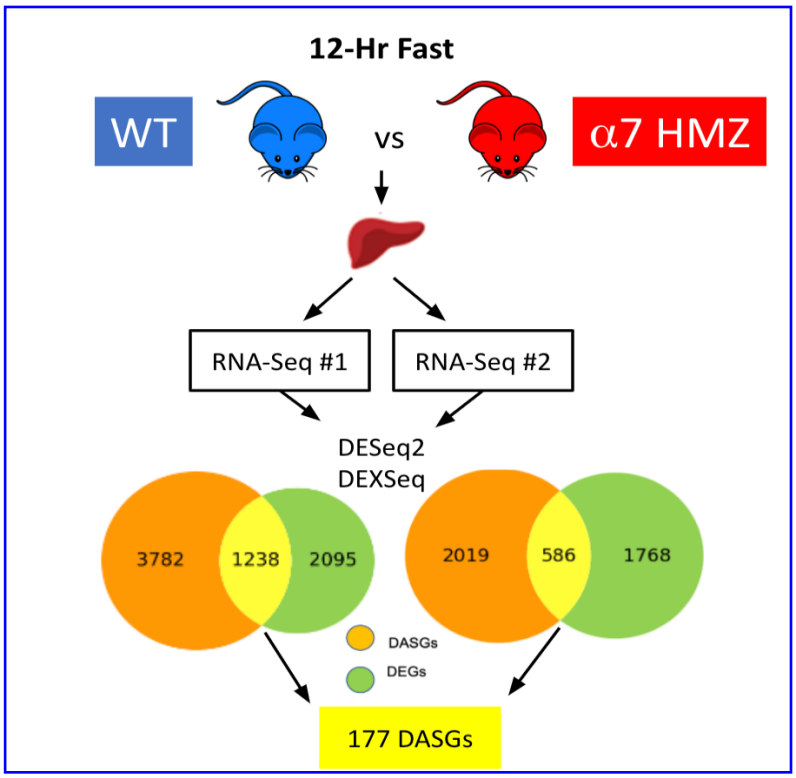

Figure 4. Experimental Design. Gene expression in livers of male mice on 12-hr fasts was analyzed by RNA-seq using DESeq2 software and alternative splicing was analyzed using DexSeq. Alternative splicing 177 genes that were both alternatively spliced (DASGs) and alternatively expressed (DEGs) in both data sets from male mice were manually curated.

Fasting HNF4a7 HMZ mice and comparing them to fasted WT HNF4a mice is important for understanding alternative splicing in metabolic disease because fasting is a metabolic stressor that induces changes in gene expression and splicing events in the liver. By comparing the alternative splicing events in the livers of these two mouse models under fasting conditions, we can identify splicing events that are specifically involved in metabolic regulation and may be dysregulated in metabolic diseases.

Following the generation of transcriptomic (RNAseq) data from the livers of male mice under several different conditions–fed versus fasted; wildtype (WT) versus α7HMZ–the RNAseq data was analyzed for differential expression (DESeq2) and splicing events (DEXSeq) (**Figure 4**). It was observed in RNA-Seq dataset #1 that the greatest number of potential splice variants was between the WT and a7HMZ mice that had been fasted for 12 hours (Martinez-Lomelli, 2022). Therefore, in the two RNA-Seq datasets, we compared the differentially expressed genes (DEGs) to the alternatively spliced genes (DASGs) in 12h-fasted mice as well as WT vs a7HMZ mice. RNA-Seq #1 identified 5,877 genes (3782 DASG + 2095 DEG) with an overlap of 1,238 genes classified as a DEG and DASG. RNA-Seq #2 identified 3,787 genes (2019 DASG + 1768 DEG) with an overlap of 586 genes classified as a DEG and DASG. We then looked at the overlap between the two triplicate RNA-seq datasets (1238 genes from RNA-seq #1 and 586 genes in RNA-seq #2, n=6) to identify 177 DASGs found in both datasets to increase the probability of identifying real splicing events.

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RESULTS

Manual Curation Criteria

Between the two RNA-seq datasets we generated, 177 genes were called alternatively spliced (DASGs). These were then manually curated using the following inclusion and exclusion criteria to identify genes with real alternative splicing events. The genes were visualized for consistency by comparing the gene body to normalized exon counts from DEXSeq using UCSC Genome Browser (provides a visual representation of the gene body, including genes of interest, regulatory regions, and other important features to explore gene expression and modifications). Genes that were marked by the program as loci where differential expression occurred were included if they fell under the following inclusion criteria and disqualified if they fell under the following exclusion criteria:

Inclusion Criteria:

1. Differential number of reads mapped to alternatively spliced exons in α7HMZ and WT
2. Alternatively spliced exons were called consistently across both datasets
3. Alternatively spliced exon has >100 normalized reads

Exclusion Criteria:

1. One genotype being consistently higher than the other across all exons
2. Values of biological triplicates vary widely
3. Small difference in normalized counts between genotypes
4. Expression levels <100 normalized reads
5. Putative alternatively spliced exon overlaps with another gene
6. Reads not mapping to known exon
7. Reads into introns

One example of a gene analyzed using these criteria is shown on the next page (**Figure 5**). DNA-directed RNA polymerase II subunit Rbp4 is a gene that met the negative criteria of one genotype being consistently higher than the other, suggesting differential expression but not alternative splicing.

The results of my study concluded that all 177 genes analyzed fell into the following disqualifying categories: small differences in splice levels (<100); putative alternatively spliced exon overlaps with another gene; RNA-seq reads that did not map to known exons; and RNA-Seq reads went through into introns of highly expressed genes. Out of 177 DASGs, there were only 16 genes that were not disqualified by the negative criteria but did not meet all the inclusion criteria to label it a splicing target (Pafah2, Ywhae, Rpl28, Itih3, Fau, Bbox1, Hpd, Gstk1, Acaa1b, mtarc2, Ndufb10, Qprt, C8g, Ugt2b36, Cyp3a11, Serpina1e). Although the results of my research demonstrated no gene fully satisfied the positive criteria to count as a significant alternative splicing event, these genes may still play a role in liver dysregulation and metabolic disease pathways that the criteria do not account for. In the fasted mice, my results also identify numerous genes related to mitochondrial function as potential splice sites. These mitochondrial genes are known to be expressed during fasting conditions and when these genes undergo loss-of-function, people can develop obesity, hyperglycemia, and hypertension.

DISCUSSION

DexSeq called over 3000 differentially spliced and expressed genes. Having two comparative data sets was helpful as it allowed for narrowing down to 177 DASGs by finding common genes in the two datasets. However, manual curation of these DASGs showed it may not be worth following up with wet bench testing. Of these, 16 genes did not fall into the negative criteria and could be followed up with alternate RNAseq programs to analyze the gene locus for any other forms of differential usage. Livers were harvested at the same

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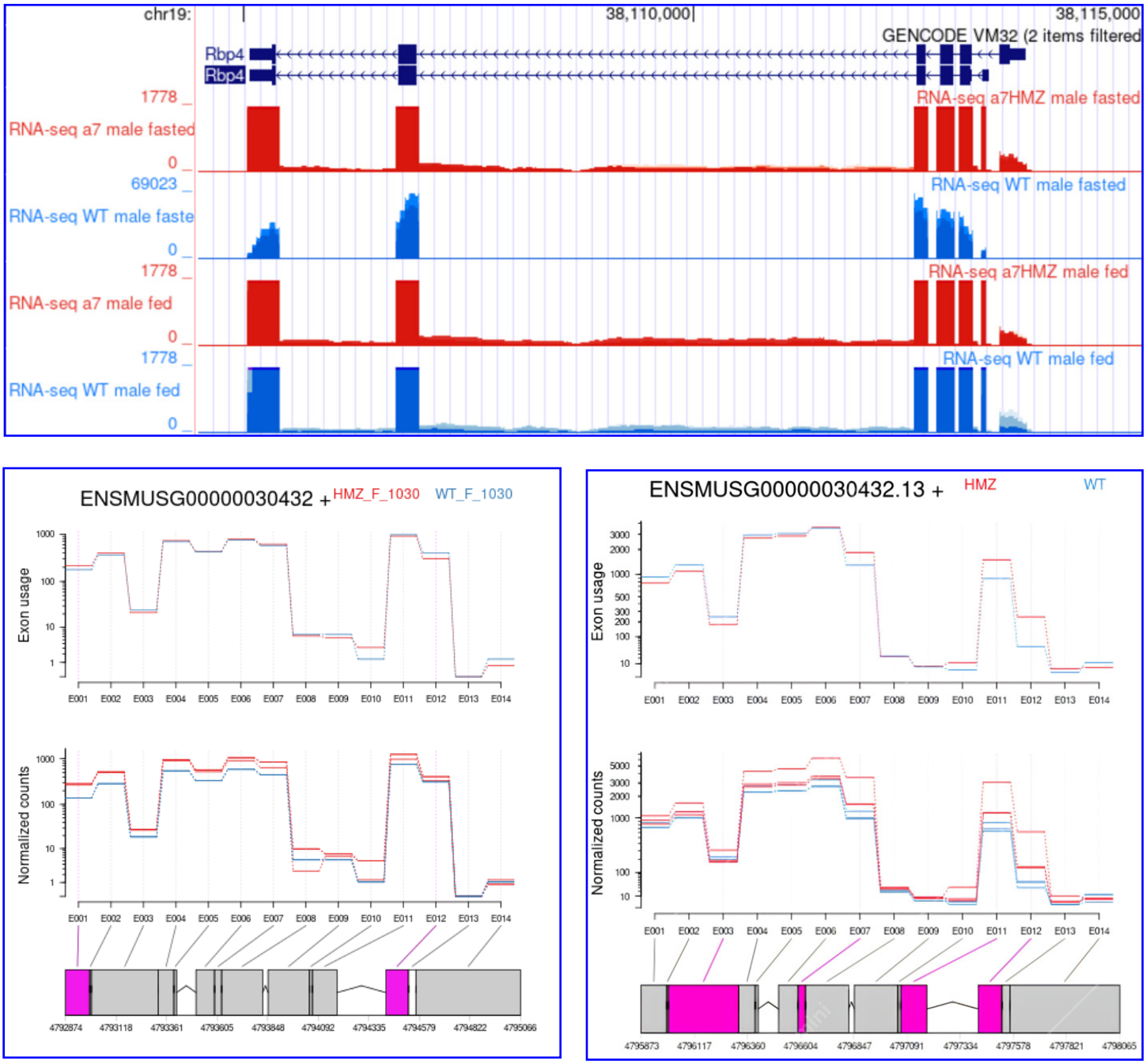

Figure 5. Example of a gene that falls within the exclusion criteria. Expression levels are displayed in UCSC Genome Browser (top) by red and blue bars of male α7 and WT mice in fasted conditions (RNA-Seq #1, 2). Gene body (top) has noticeably fewer exons than shown by Dexseq maps (bottom), making mapping challenging; Red circled exons: called alternatively spliced across both datasets by DexSeq. Expression levels are <100 normalized counts, falling under the significance cutoff.

time of day following the same fasting regime, so differences in the two DexSeq-generated maps for the two RNAseq datasets may be a result of the background or strains of the mice in the two datasets as well as any RNAseq noise. Different sequencing protocols were used in the two RNAseq datasets: 75-bp single-end versus 150-bp paired-end which could account for some of that difference. Current RNAseq programs do not account for all the different variables that could go into making an accurate alternative splicing call. DexSeq was selected for this purpose because it gave the greatest number of calls, but future research could use alternate programs to analyze gene targets more accurately.

While aligning the DexSeq-labeled exons to exons on the gene body in UCSC Genome Browser, there were differences in the number of exons identified and the orientation of the gene. This made aligning these exons difficult. This was exacerbated by exon maps in DexSeq not coinciding with the orientation of the gene in UCSC Genome Browser. The program called many mitochondrial genes usually expressed during fasting conditions and implicated in obesity, hyperglycemia, and hypertension. With this research, we were able to support a role for alternative splicing in metabolic disease that should be followed up with wet-lab testing. Further research could be conducted to analyze the roles of those mitochondrial genes in similar metabolic disease models.

Additionally, with intermittent fasting being a recommended intervention to improve liver health in patients with metabolic disease, studying the role of the liver master regulator, HNF4A and its isoforms with transcriptomics data from mice as done in our study can clarify essential molecular and cellular mechanisms that regulate liver metabolism during fasting and feeding (Bolotin et al., 2019; Lu et al., 2016). Previous studies have shown intermittent fasting alters HNF4a activity (Hatchwell et al., 2020). Thus, studying subsequent gene expression changes using exon-swap mice and RNAseq data could clarify the gene expression pathways by which new diet interventions address metabolic disease and liver health to inform preventative nutrition changes or drug therapies.

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The Mahsa Amini Revolution

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ABSTRACT

The objective of this paper is to explore what occurred during the 2022 Iranian revolution by comparing how the event is portrayed by American media sources and the testimony of an Iranian American Woman. The paper then explores the relation between women's bodily autonomy and state governments such as the Iranian and French governments who either enforce or suppress the practice of veiling through law. This paper concludes that American Media portrays the 2022 Iranian revolution from an Islamophobic perspective. This paper offers alternative views in which the practice of veiling and the topic of women's bodily autonomy can be viewed in relation to Islam. Additionally, this paper claims that in addition to Iran, Western countries also inhibit the bodily autonomy of women.

KEYWORDS: Islam, Religion, French Law, Islamophobia, Veiling, Hijab

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The Mahsa Amini Revolution

POINT OF DEPARTURE: UC STUDENT WORKERS SUPPORT EFFORTS IN IRAN

Beginning Monday, November 14, 2022, 48,000 graduate students, along with numerous staff and undergraduate students, began striking against unfair labor practices across the UC system. During this strike, hopes were high and chants were both humorous and uplifting: "Get up! Get down, Riverside is a union town!" Communities gathered, sharing resources to feed the strikers and provided much-needed support to the exhausted student workers. On November 30, organizers at UC Riverside decided to dedicate the day to the Iranian Student Association and hold a protest regarding recent events which were unfolding in Iran. Chants such as "Beat me! Hit me! You can never break me!", "It's not about my hair; it's about my voice! It's not about my body; it's about my choice!" rang throughout the campus. Other than seeing a brief news report through social media, for many students this was the first time they encountered the Iranian protests in reaction to the death of Mahsa Amini, dubbed the Mahsa Amini revolution. Suddenly, the strike was not just about the treatment of local underpaid and overworked college students. The strike carried a weight much greater than wage issues; it carried a desolate cry for action, "Iranians made their choice! America be their voice!"

The following paper will discuss the recent Mahsa Amini revolution and achieve the following three goals: discuss how the event is portrayed and misunderstood by popular US media sources, reframe the event in relation to women's rights and Islam, and discuss how Western nations fail to protect women's rights in contrast to US media's claims that Islamic nations fail to protect women's rights.

THE REVOLUTION THROUGH THE EYES OF AMERICAN MEDIA

On September 22, 2023 a 22-year-old woman by the name of Mahsa Amini died while in the custody of Iranian authorities. According to Iranian authorities, Amini was arrested and placed in custody as a result of violating a public veiling mandate, which obligates women living in Iran to cover their heads and hair while in public spaces. Laws such as these are influenced by social regulations discussed in religious scriptures such as the Quran and other texts recognized as canon by the Iranian government. These religious scriptures are often characterized and criticized by Western news outlets as being inherently repressive and misogynistic. Western news outlets have coined the term, "morality police" to describe the authorities who regulate women's bodies in Islamic state governments (Karadsheh and Qiblawi, 2022). Media sources reported that the Iranian "morality police" unjustly arrested Mahsa Amini and physically abused her while she was in custody, eventually leading to her death. For the Iranian public, this was the last straw as many women have been subjected to harassment and physical abuse by Iranian authorities. Within hours of the news about Mahsa Amini, protesters took to the streets in solidarity with her and the many women who have faced similar fates. Chants similar to those at the protest held at UC Riverside could be heard throughout the streets of Iran: "زن زندگی آزادی" which translates to "Women, Life, Freedom." Videos taken by protesters in Iran show female university students publicly taking off their veils in protest of the regime, police dragging women by their hair through the streets, and paramilitary tactics being used against civilians. In addition to police brutality, attempts have been made by the Iranian government to prevent information from being shared by instituting nationwide internet outages.

In addition to the injustices enacted on women's bodies, these protests are in reaction to a number of social restrictions put in place by the Iranian government during the last few decades. US news outlets correctly reported that Mahsa Amini was unjustly murdered by an authoritarian government and that there was an outbreak of civil disobedience in solidarity for Mahsa Amini as well as in reaction to ongoing

political conditions. Where US media falls short, however, is its portrayal of Iran as a nation which has always been oppressive as a result of extreme religious ideation. There was no discussion of the history of Islamic law discussed nor of the conditions the led to the creation of an authoritative theocracy. Instead, news outlets pushed an easy and dirty narrative which has become all too familiar to US audiences over the last two decades. US media failed to portray the multidimensional aspects of Iranian society in which women have differing levels of freedom dependent on their social status, economic status, or geographic location in the country. US media also did not inform the public about historical instances of Islamic societies promoting better conditions for women and minority groups. Nor did new outlets discuss the nuanced ways in which political authoritarianism has weaponized religious values.

Building off of fear-mongering and Islamophobia, news articles made outlandish claims, including comparing Iran to North Korea. Many reports argued that Iran's potential for nuclear weapons should be cause for US attention, while overlooking that the government's human rights violations should be enough for international attention: "The only thing worse than a regime that kills and represses its own people is a regime with a nuclear weapon and that kills and represses its own people" (Karadshen and Qiblawi, 2022). Statements such as these give rise to concerns about the intentions of US news outlets. What relevance does the potential for nuclear weapons have to this story? Why would the authors quickly add this point at the end of their report when it does not seem to fit in with the rest of the theme?

Typing the word "Iran" into the popular video-sharing platform YouTube immediately returns videos with titles such as, "US-Iran conflict escalates" by ABC News, and "Iran-backed forces ramp up strikes on US bases in Syria" by Fox News, or "Inside Iran's Raging War on Drugs" by Vice. Scrolling through YouTube's recommended videos based on the keyword, "Iran" shows many dramatic and fear-provoking titles such as these, with only a few positive titles such as, "Undiscovered Iranian Street Food Tour" by Mark Wiens sprinkled in. With many US citizens now using platforms such as YouTube as a source for news, it is alarming how quickly the platform distributes negative information against Islamic nations such as Iran.

One CNN interview titled, "Iran is Raising the Bar: Ex-NATO commander on the attack against US troops in Syria" asked General Wesley Clark to further comment on the US and Iran's involvement in an ongoing conflict in Syria. General Clark claimed that drones manufactured in Iran have been used to strike US military personnel. The general also claimed that the US does not wish to escalate the situation, but it is prepared to assume a defensive position if needed. General Clark then speculates what Iran might intend to do in this situation and suggests that Iran's goal is to keep Israeli and US troops from organizing in the area so they may take up a more advantageous position and push US presence out of the region; "It's all a part of Iran's quest for regional homogeneity" (CNN, 2023). CNN does not ask what General Clark means by "regional homogeneity" and viewers are left unclear if he meant an Iranian homogeneity of political ideals or cultural identity. CNN reports also do not ask him to comment on the US's intentions in the region. It becomes clear how media outlets such as CNN provide an oversimplified narrative of a complicated conflict in a way that creates a heightened sense of Islamophobia within the American public.

As easy as it is for US media to criticize the Muslim world for inhibiting the bodily autonomy of women, they fail to recognize the ways in which Western society also inhibits the bodily autonomy of women, including anti-veiling laws being passed in nations such as France. US media utilizes the fear of impending or ongoing war by limiting what military generals have to say regarding the ongoing conflict to further paint Arabic culture in a negative light. They also fail to contextualize the complexities of Islamic culture and

historicize Islam's relation with women's autonomy over time. By leaving out these important perspectives, US media misinforms the public and continues to spread Islamophobic ideology in a nation which claims to be founded on the notion of religious freedom.

THE REVOLUTION THROUGH THE EYES OF AN IRANIAN-AMERICAN WOMEN

One Iranian student from UC Riverside, Dina Mirmotalebisohi, has commented on the situation, describing how Islam is a religion that has the potential to be beneficial for society. Rather, it has been used by groups such as the Iranian government to oppress people rather than liberate them. "You can never understand how much they're hurting" (Mirmotalebisohi, 2023). She describes how oftentimes as a result of the Iranian government's policies, people who emigrate from Iran to the US often choose to no longer practice Islam. If they do choose to practice Islam, they do so on their terms rather than how it is prescribed by the Iranian government. She recalls her own mother's life as an immigrant, stating how her mother simply needed to escape said environment. Her mother's struggles did not end with leaving Iran; her mother continues to worry for parents, siblings, friends, and relatives who are still suffering under the Iranian government's policies.

Regardless of the political climate, Mirmotalebisohi affirms that Iranian culture is beautiful, she recounts her time spent with family there stating how lively the environment is, "It's [exciting] and fun, like, there's all that beautiful laughing energy. Family is huge. When I'm [in Iran], I'm ready to be around family 24/7" (Mirmotalebisohi, 2023). Mirmotalebisohi also recounts how in Iran there is diversity regarding the strictness of veiling practices throughout the nation, contrary to how American media portrays the country as homogeneously appealing to old and outdated ideology. In reality, there is diversity regarding the strictness of veiling practices throughout the nation. Only in cities under strict government control, such as Teheran, are women in danger of persecution. Mirmotalebisohi says this is because of the way social structures function in the cities, that areas with larger government presence are more restrictive. As we can see through this discrepancy, it is not Iranian culture which aims to persecute women but rather the Iranian government. If veiling were not mandatory, it would likely be the case that while many women would enjoy the freedom of not being legally obligated to veil, others would likely continue to veil whether for religious or cultural reasons. This is contrary to how American media has portrayed the climate of the country which shows how American media tends to give a biased account of a multidimensional social and political climate rather than giving a fair perspective of the entirety of the country's state of affairs.

She recounts how within the homes of Iranian citizens, Iranian women are talkative, confident, and outgoing. She comments how contrary to how American media has portrayed them as being weak and in distress, they are brave and are ready to stand up for themselves and demand respect within their society. Further commenting on the bravery required to do so in Iran, Mirmotalebisohi describes how the risk to do so in Iran is significantly greater than in the US. Since the government is authoritarian and has a monopoly on violence, it can directly silence those who publicly oppose them with little to no accountability. Although we are aware of many women who have faced violence from the Iranian government, the actual amount is undoubtedly much higher due to the government's ability to hide their actions. Additionally, it is the fear of violence from the government which makes it difficult for women or men to publicly display their disapproval of government policies. It is conditions such as these which cause tensions to build to a point in which citizens are frustrated enough to resort to mass civil disobedience such as in the Mahsa Amini revolution.

When asked how much of the conflict is due to political, social, or religious factors, Mirmotalebisohi states that

the conflict is overwhelmingly due to political policy and that social and religious factors play only a small part in the tension being built. This may come as a surprise to an American audience as the Iranian government is a theocracy. However, as Mirmotalebisohi states, it is the political ideology that drives violence against women, that scripture has been manipulated by corrupt officials to serve their purposes rather than being used to serve and liberate people.

Commenting on how people have been attempting to help the situation in Iran, Mirmotalebisohi is concerned about how people utilize social media in unproductive ways. She notes that some social media users are quick to critique those who do not publicly post about the Mahsa Amini revolution; however, it could easily be the case that a person may not feel that it is safe for them or their family to have a public opinion about the topic. Additionally, Mirmotalebisohi points out the hypocrisy of those who frequently post about the situation but refuse to speak about it in person, go to public demonstrations, or donate funds to the cause. "I think there's more to it than just social media. And we often get that confused, because social media is really easy [to use an end all be all], at least for some people" (Mirmotalebisohi, 2023).

Mirmotalebisohi explains that the Iranian Student Association held a protest in support of those revolting in Iran so they could see that there are people in the US who empathize with their struggles. The Iranian Student Association also wanted to raise awareness of Iranian immigrants and their descendants who are struggling in the US as a result of the conditions in Iran. However, because the issue is related to veiling as a religious practice, it becomes both difficult and nuanced to accurately critique the violent enforcement in Iran without the possibility of one's message coming off as more aggressive than intended. "It's hard to yell on campus 'Down with the Islamic regime!' What we mean is 'Down with the Iranian Islamic regime' right? But what people hear is 'Down with Islam!' We're not saying we hate hijabs. We're saying we hate the force that's associated with wearing a hijab in Iran. We're saying that we're against taking away [the bodily autonomy of women]" (Mirmotalebisohi, 2023). In addition to the violation of women's rights, Mirmotalebisohi notes that while men are able to exist and live in accordance with behavioral standards prescribed by the government, albeit in better conditions than women, men in Iran also have a difficult time navigating the regime's regulations. Furthermore, those who belong to social minority groups, such as LGBTQ persons, or members of racial minority groups, such as Mahsa Amini who was Kurdish, are more likely to have a difficult time and face neglect as well as harassment from the government. The perspectives of these groups are often overlooked by US media.

REFRAMING VEILING THROUGH A HISTORICAL LENS

According to author Reza Aslan, there is little doubt that the Quran as a scripture was shaped by the surrounding culture for which it was written (Aslan, 2012). Many societies in the past, both Western and Eastern, conceived women as being less of a person than men, which may explain why there are several lines in the Quran seemingly asserting misogynistic values. Aslan argues that it is these parts of scripture that are weaponized by groups such as the Iranian government to justify the policing of women's bodies. Aslan argues that if one only selects specific parts of the Quran, one could call for almost any form of government from a neo-liberal form of capitalism to a hyper-Marxist form of communism. If one looks at the Quran as a whole, however, it is evident that the Quran argues for an egalitarian society free of social disparities where religious institutions and private individuals pool resources to promote the well-being of marginalized groups, even those who do not practice the Islamic faith. Furthermore, Aslan highlights how the Prophet Muhammad promoted the inclusion of women in public and social life by not discouraging his wives from participating in communal prayers and traveling to places of prayer without a male escort. This is contrary to how the Quran

is interpreted by the Iranian government which prohibits women from traveling to public places of prayer without the escort of a close male relative. Aslan then elaborates how while the Iranian government attempts to police women's bodies and restrict their choice to veil or not to veil, Western governments are guilty of the same issues (Aslan, 2012).

Sofia Rehman, a scholar specializing in Islam and gender, also discusses the Prophet as a model for behavior in Islamic practice. She points out that while it is a nice thought to argue that the Prophet was inherently feminist and directly argued for the equality of women, this is an oversimplification of reality. One must accept how The Prophet did live in a patriarchal society and as a result, he exhibited patriarchal behaviors. Contrary to what traditionalists argue, the Prophet playing into his role as a male-bodied person in a patriarchal society does not necessarily imply that the Prophet advocated for a patriarchal society, nor a society whose state violently enforces veiling onto women. Rehman elaborates on how the most accurate view is to create a synthesis between these two perspectives. While the Prophet existed and functioned in a patriarchal society, his ultimate goal was to reform it to be more inclusive and welcoming of those who were considered lower class within said context. We see that the Prophet advocated for women's agency in areas such as marriage and divorce by arguing that women should be able to have the opportunity to provide verbal consent to marriage. Additionally, the Prophet argued for the fair treatment of young women who lost their legal guardians due to unforeseen circumstances. Since many instances of the Prophet resisting patriarchal structures exist in the Quran, further arguments can be made in favor of the progression toward an egalitarian Islamic society. For if the Prophet truly envisioned a completely patriarchal society, he would likely have not advocated for better marriage and divorce conditions in marriage nor would he have advocated for better treatment of women without legal guardians (Rehman, 2022). While this aspect of Islam is evident to many scholars, it is often ignored by US media, which continue to portray countries such as Iran as primitive and lacking understanding in contrast to the West.

Contemporary veiling issues exist outside of Iran, including laws passed by France's ultra-secularist government that target Muslim women and prevent them from veiling in certain spaces, regardless of the women's own choices. Such laws are based on philosophy from the Enlightenment period in Europe which was meant to promote women's liberation. The French term laïcité describes a specific type of radical state secularism based on the 1905 Act on the Separation of Church and State which declares that the French government will not recognize or monetarily endorse any form of religion. This notion was then incorporated into Article 1 of the 1958 French Constitution stating that France ought to be a secular and social Republic. This form of radical secularism was meant to promote public and social equality and prevent the abuse of state powers to favor one religious group. Instead, it has been weaponized to disenfranchise religious groups and promote state-sponsored Islamophobia, which while it is not the promotion of religion, certainly does not constitute as religious neutrality.

We can see numerous examples of state-sponsored Islamophobia in France. In one famous 1989 case, three adolescent girls were expelled from a French school because of their refusal to remove their veils when prompted by the school's administration. This event became known as the Creil Affair, and the incident eventually made it to a French court (Baldi, 1970). The court ruled that certain acts or symbols intended to display religious affiliation were not necessarily incompatible with *laïcité* as long as the act or symbol was part of the individual's religious expression. While the court did rule in favor of the use of some religious symbols, symbols that could be considered propaganda, vulgar, or provocative could still come under legal scrutiny. Many Muslim women continued to face scrutiny for attempting to receive an education from French school while veiling in classes. In 1995, French courts made a contrary

proclamation that veiling was to be considered out of line with the notion of *laïcité;* small crosses or small star of David emblems were still permitted. This made it acceptable to legally prevent students from veiling in French schools.

As noted earlier, the French courts have struggled with deciding if public veiling is a violation of *laïcité*. Part of the confusion appears to be whether the veil is considered a religious or political symbol. Baldi argues that the French government bans veiling without any sound logic or use of empirical evidence. Citing how French courts view veiling as inherently enforcing the alienation of women and claim that the practice of veiling is only ever done through coercion and by forceful means. This type of thinking exemplifies dismissive attitudes towards other cultures which may simply have different values as French culture. The depiction of Muslim women as inherently passive and in need of saving from their own culture is both appalling and is a one-dimensional outlook on the complexities of Arab culture (Baldi, 1970). According to author Reza Aslan, "at the heart of this argument is the profoundly misogynistic belief that no Muslim woman would freely choose to wear the veil. [...] That women are incapable of deciding for themselves what they should or should not wear" (Aslan, 2012). This highlights a deeply rooted flaw in the thinking of the French government in that they seem to believe that Muslim women are incapable of deciding for themselves if veiling is good for them. Additionally, the argument that creating a legal obligation to not veil in public does not make sense in the context of the Western definition of dignity, being the ability to autonomously decide for oneself. Given this perspective, anti-veiling laws should be viewed as undignified through the eyes of Western thought.

Another example of French society inhibiting women's freedom of choice is the Burkini Affair of 2016. Approximately 30 small towns in France passed local laws which prevented Muslim women from wearing a type of one-piece swimsuit that covers the entirety of the body including the length of one's arms and legs as well as a hood that covers one's hair, leaving only the face, hands, and feet exposed. This ban was enacted on the ground that the burkini was thought to be a symbol of Islamic extremism. These laws were fought in court and were only upheld by several courts only to be finally overruled by the French Conseil d'État which declared the ban a violation of fundamental freedoms (Baldi, 1970). We can see through these examples how the veil began as a cultural marker before becoming a tool for governments, whether Islamic or Secular, to control women's bodies and inhibit their autonomy as free subjects (Baldi, 1970).

CONCLUSION

The Mahsa Amini revolution occurred as a reaction to the unjust treatment of women under Iran's political policies. US media was quick to present this event as a sign that Islam is inherently misogynistic and used it to portray Iranian society as backward. They did so only by presenting an incomplete version of the larger issue at hand and forgoing the inclusion of veiling's historical context. By doing so, US media outlets presented only the negative aspects of veiling as a form of misinformation. The lack of historical context allowed US media to peddle the myth that the spread of Islamic society is inherently a threat to US national security, an ironic assertion from a nation that boasts about its acceptance of religions.

The perspective of Iranian-American women makes it clear that the policing of women's bodies is not inherent to Iranian culture, and that Iranian culture is as modern as any other. Iranian women are both vocal and ambitious in their efforts to achieve equality within their own society. Looking at the history of Islam, it is clear that religion was meant to promote egalitarianism and the autonomy of women rather than the oppression of women through state-sponsored patriarchal laws. It is bad government bodies who take scripture and utilize it to promote their independent ideals rather than religion and culture which seeks to further oppress women

as a social class. Through this perspective, it becomes evident that the policing of women's bodies is due to bad government bodies rather than bad religion.

Finally, we see that it isn't only Islamic governments who are capable of utilizing ideas that were meant to liberate people and use them to oppress citizens.-Western countries such as France are also guilty of policing women's bodies by preventing them from having the choice to veil in public. This has been done through the manipulation of the French constitution to create anti-veiling laws such as the Burkini Affair of 2016 as well as many instances where women were prevented from participating in the public education system for refusing to remove their veil. While the French government claims they do so in the name of Women's liberation, it is clear that their true intention is to disempower Muslim citizens which in no way should be considered religious neutrality.-

Following these conclusions, there is much research that must be conducted to further understand the Mahsa Amini revolution. This paper discussed how American media outlets such as popular news websites have covered the issue in addition to how popular search engines such as YouTube present the nation through their search query. This does not cover the vast amount of American news sources such as independent news, television, or American social media platforms such as Facebook, Instagram, or Twitter. Nor does this paper explore how Chinese social media platforms such as TikTok allow the situation to be presented on their platform. In addition to further analysis of how different media outlets present the situation, further research ought to be conducted in regard to how other Iranian voices perceive the Mahsa Amini revolution. Although this paper interviews an Iranian-American woman regarding the situation, there is a plethora of different perspectives which may be had by Iranian or Iranian-American women depending on their social-economic background and cultural affiliation. Finally, further analysis should be conducted to conclude how it is that the Iranian government interprets the Quran currently and how the Quran has been interpreted historically.

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Less is Less: Fast Ad Delivery Undermines Impact

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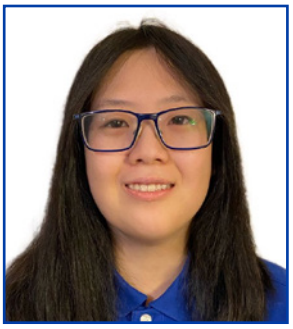
ABSTRACT

In the digital media age, video advertisements are ubiquitous, including on platforms such as YouTube and TikTok. People generally do not like advertisements, especially non-skippable ones, and may prefer to do something else while an ad plays. Following recent research suggesting that faster speeds may increase engagement with videos, we examine if speeding up video advertisements will increase people's attention when watching non-skippable ads as well as improve ad experience and memory. In two experiments, college participants were randomly assigned to watch an ad (Vrbo in Study 1, Five Star Notebooks in Study 2) at either normal or 25% faster (1.25x) speed. We did not find significant effects of playback speed in Study 1, perhaps because the ad did not have much informational content. However, in Study 2, we found that participants who watched the ad at 1.25x speed enjoyed it less, remembered it less well, and had lower brand sentiment than those who watched it at normal speed. While these findings need replication and further research to test robustness across a range of ad types and lengths, our results suggest that increase the speed of ads may not be beneficial to advertisers.

KEYWORDS: video speed, advertisements, brand sentiment



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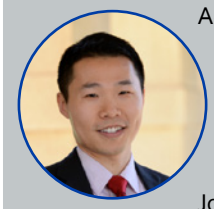

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Less is Less: Fast Ad Delivery Undermines Impact

INTRODUCTION

In the digital media age, video advertisements are ubiquitous. They are often non-skippable and are an obstacle to endure to get to the content people are actually interested in viewing. Given recent research (e.g., Duan and Chen, 2019; Lang et al., 2020; Nagahama and Morita, 2017) suggesting that higher video speeds have a positive impact on the viewers engagement with videos, we wondered if the benefits of speeding up video playback might apply to advertisements as well. In particular, will a faster advertisement be more engaging to people, leading to greater attention toward and enjoyment of the ad, and subsequently to better memory of the ad and improved brand sentiment?

BACKGROUND

Playback Speed

The effect of playback speed on people's experience watching videos has been studied in a variety of fields as diverse as education, entertainment, and marketing. One prominent area of research is in education, where there is the natural question of the relationship between video playback speed and student learning outcomes in Massively Open Online Courses (MOOCs). For example, Lang et al., (2020) found that only one-fifth of MOOC learners ever modified video playback speed, which suggests that many users may be unaware of the option to change the playback speed or at least do not do so habitually. The median user who did change speeds tended to choose either 1.25x or 1.5x speed. In an experimental study, researchers randomly assigned learners to have their default video set to either normal speed or 1.25x speed. Although learners could change the video speed themselves, they would have to manually adjust it every time they watched a new video. The study found that the 1.25x speed group took less time to complete the MOOCs, which translated into higher course completion rates and persistence. Lang et al.'s (2020) findings are consistent with related work showing that students assigned to watch lecture videos as 1.5x speed scored higher on comprehension tests than those assigned to watch at 1x speed (Nagahama and Morita, 2017), although their sample size was fairly small (n = 59). Students in the study assigned to watch lecture videos at 2x speed did not like doing so and did not perform better in the course. Another study found that lecturers were perceived to be more credible and authoritative when speaking with a faster cadence (Simonds et al., 2006). However, not all studies show a positive benefit of faster educational videos. For example, Ritzhaupt et al., (2015) found that video speed had no effect on learners' performance and that learners were more satisfied with normal video speed than with other video speeds. Notably, this study is somewhat older than the other papers, so it is possible that students have become more used accustomed to faster video speeds in more recent years as the option has become more common, with built in settings on platforms such as EdX, Coursera, YouTube, and even Netflix.

Another study done on playback speed watching entertainment videos online explored why a viewer would choose to increase a video's speed. Duan and Chen, (2019) surveyed 242 Chinese college students who reported watching dramas on streaming platforms and found that 84.3% of participants watched streaming dramas above 1x speed. Among 12 participants selected to partake in in-depth personal interviews, reasons reported included increasing time efficiency, preference for a fast-paced lifestyle, and as a remedy for plot dragging. This is consistent with recent work by Li, (2022), which found that viewers of infotainment videos found them more enjoyable when viewed at 1.25x speed (compared to 1x speed) and reported paying more attention to them.

Advertising Effectiveness

Companies spend significant amounts of money on advertising and much of this budget goes toward video ads whether on television or the internet. Companies, therefore, are likely to be interested if people actually watch their ads and, ultimately how effective those ads are in terms of

increasing people's brand sentiments and memory of the ad content. For example, a study on advertisement effectiveness by Bellman et al., (2020) examined whether viewing time had a nonlinear relationship with ad effectiveness. It found that as the amount of time an ad is viewed increases, recall, recognition, ad liking, and purchase intention increase, but with diminishing returns.

Advertising researchers have started to examine the effects of advertisement speed. For example, Bolls et al., (2003) examined the effects of advertisement pacing on viewers' voluntary and involuntary attention to an ad and its effects on the viewers memory of the ad. Pacing refers to the number of visual cuts in the video, so not exactly speed, but a related concept. They found that while there may be some evidence that fast-paced ads have positive effects on the viewers' involuntary attention (arousal), that attention appears to be directed towards the "advertisement execution" part of the ad and not its actual message. That is, participants paid more attention to the ad but had worse recall for the ad's message. In a related study, Sundar and Kalyanaraman, (2004) also found that faster-speed animations in ads were better at holding participants' attention but also found that slower speeds seem to enhance the advertised website's appeal. These studies suggest that faster speeds may increase attention but have unintended consequences for both liking and remembering the ads.

Hypothesis

In summary, studies in a variety of domains have found that faster video speeds increase viewers' engagement and attention. Studies in education and entertainment seem to suggest that faster ads also lead to greater performance and enjoyment, respectively. However, studies on ad pacing and animation speeds seem to suggest that this increased attention may not confer benefits in terms of brand sentiments and ad memory. We therefore hypothesize that increasing the speed of the ad will increase people's attention towards non-skippable ads. However, we are less certain if faster speeds will increase ad enjoyment and brand sentiments. Finally, we hypothesize that faster speeds will reduce ad memory.

STUDY 1: VRBO

To test the effect of playback speed on advertising effectiveness, we conducted a study in which participants watched a non-skippable advertisement at normal or 25% faster speed, and then the participants on their recollections of the ad and asked them about their experience watching the ad as well as their brand sentiments.

Methods

Participants: We recruited 174 college students from the University of California, Riverside (76 females and 98 males) to complete a series of studies in exchange for partial course credit, of which this study was one. The average age of the participants was 21.2 (SD = 2.26, range = 19 to 36; 55.1% Asians, 26.3% Latino/Hispanic, 8.0% White/Caucasian, 1.7% Black/African, and 6.8% other ethnicities).

Video: We used a 60-second-long Vrbo advertisement from the 2022 Super Bowl hosted on YouTube. We chose Vrbo because it is a well-established company, but less known than companies like Apple or Samsung. This would help minimize participants' preconceived notions regarding the company that could reduce the impact of the ad on participants' brand sentiment.

Questionnaire: The questionnaire was designed in Qualtrics, a web-based survey platform. We created a series of questions to ask about participants' experience watching the advertisement (see **Table 1,** next page). Participants rated their agreement with statements on a Likert scale from 1 to 7, with 1 being "Strongly Disagree" and 7 being "Strongly Agree." There was also a four-item quiz about the advertisement's content to measure the participant's memory of the ad.

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Variable	Statement Wording
Enjoyment	I enjoyed this video.
Entertainment	I found this video entertaining.
Bad Experience	Watching this video was a bad experience.
Informative	I found this video informative.
Attention	I paid full attention to the entire video.
Understanding	I fully understood the content of this video.
Relaxing	I found this video relaxing.
Stress	I found this video stressful to watch.
Ad Message Understanding	The ad message is understandable.
Ad Relevance	The ad's message is relevant to me.
Ad Believable	The benefits described in the ad are believable to me.
Product Usage	After viewing this ad, I would consider using the product.
Brand Sentiment	I feel positively toward the brand in this ad.
Perceived Speed	The video playback felt faster than normal.
Audio Distortion	The audio in the video seemed distorted or weird.
Video Distortion	The images in the video seemed distorted or weird.

Table 1. Likert Survey Questions (1 – 7 agreement scale)

Procedure: Participants were randomly assigned to the normal video speed group (1x) or the 25% increased speed group (1.25x). They first watched the video advertisement at the assigned speed, with no option to change the speed or to skip, pause, or rewind the video. The page automatically advanced to the next page once the video was done. Participants then answered the Likert-scale questions about their experience watching the advertisement and about their brand sentiments. Finally, they completed the four-item multiple-choice quiz to test ad memory. We also asked about participants' demographics and typical behavior towards video advertisements outside this study.

Ethics Statement

Electronic informed consent was obtained from all participants at the start of each study.

Results

We used Jamovi v.2.3 (2022) to analyze our data. Table 2 shows the results of t-tests between conditions as well as linear regressions of the effect of video speed for all dependent variables. We created scores for the memory task by totaling up the number of correct responses out of four on the multiple-choice quiz. The group mean for the normal speed group was higher than the increased speed group for all of the dependent variables listed except for enjoyment and entertainment.

Table 2 shows the results of t-tests between conditions as well as linear regressions of the effect of video speed for all dependent variables. We also included gender, age, and prior familiarity with the brand as control variables as these might impact prior preferences for

Variables	*M*		*SD*		*t*-stat	*p*	*Regression*		
	1x ($n = 89$)	1.25x ($n = 92$)	1x	1.25x			*b*	*t*-stat	*p*
Enjoyment	2.17	2.40	1.06	0.83	-1.64	.10	0.27	1.97	.05
Entertainment	0.64	0.84	1.21	0.98	-1.21	.23	0.23	1.43	.15
Bad Experience	-0.97	-1.23	1.09	0.99	1.70	.09	-0.29	-1.95	.05
Informative	-0.09	-0.23	1.14	1.08	0.84	.40	-0.10	-0.59	.56
Attention	1.38	1.35	0.87	0.84	0.27	.79	-0.03	-0.23	.82
Understanding	1.06	0.79	0.96	1.01	1.79	.08	-0.24	-1.63	.11
Relaxing	0.58	0.33	1.03	1.07	1.65	.10	-0.22	-1.39	.17
Stress	-1.31	-1.12	0.95	1.06	-1.31	.19	0.19	1.23	.22
Ad Message Understanding	0.93	0.73	1.05	1.10	1.28	.20	-0.17	-1.08	.28
Ad Relevance	0.19	0.14	1.18	1.10	0.29	.70	-0.01	-0.06	.95
Ad Believable	0.49	0.30	1.03	0.95	1.29	.20	-0.17	-1.16	.25
Product Usage	0.24	0.16	1.08	1.05	0.46	.65	-0.03	-0.20	.84
Brand Sentiment	0.57	0.44	0.98	0.86	0.97	.33	-0.12	-0.82	.42
Perceived Speed	-0.33	-0.11	1.26	1.33	-1.13	.26	0.22	1.13	.26
Audio Distortion	-1.10	-0.97	1.12	1.12	-0.80	.42	0.11	0.66	.51
Video Distortion	-1.21	-1.14	1.08	1.01	-0.46	.64	0.07	0.46	.65
Quiz Score	2.53	2.15	1.45	1.93	1.48	.14	-0.30	-1.22	.22

Table 2. Descriptive Statistics for Dependent Variables, *t*-tests, and regressions of Normal vs. Fast Groups

Vrbo. Although these control variables had significant effects for some dependent variables, we do not report these effects because they are not the focus of our research.

We found marginally significant effects of faster speed on enjoyment (b = 0.269, t = 1.97, p = 0.050) and bad experience (b = -0.293, t = -1.96, p = 0.052), suggesting that participants enjoyed watching the ad somewhat more at 1.25x speed than at 1x speed. However, faster speed did not have a significant effect on any of the other dependent variables that we tested. For example, faster speed did not have a significant effect on the memory quiz, but the findings were in the direction of worse memory (b = -0.304, t = -1.220, p = 0.224). Similarly, faster speed did not significantly impact self-reported attention for the video (b = -0.030, t = -0.23, p = 0.816), its informativeness (b = -0.098, t = -0.59, p = 0.556) stressfulness (b = 0.186, t = 1.23, p = 0.221), or sentiments toward the brand (b = -0.108, t = -0.82, p = 0.415). Although none of these effects were significant, the direction of these effects was consistent with a negative impact of faster speeds with the exception of enjoyment, entertainment, and bad experience.

Discussion

For this study, we did not find many significant differences between the normal speed and increased speed groups. Although participants marginally enjoyed the ad more, the direction of the other insignificant effects suggest negative impacts of faster speeds on the ad's stressfulness and effectiveness as well as viewers' recall of the ad content. The lack of significant effects may in part be due to the smaller than expected sample size because the class we drew participants from had fewer students than expected. It is also possible that the Vrbo ad—which consisted of relaxing music playing over videos of family vacations—perhaps had too little content or was irrelevant to student participants. Finally, the memory quiz we used may have been too blunt an instrument to distinguish between different degrees of ad memory.

STUDY 2: FIVE-STAR NOTEBOOKS

To address the limitations of Study 1, we changed the ad to one that was more geared toward students. We also added a free-recall memory task to directly measure recall memory in addition to the cued memory that multiple-choice questions measure. Additionally, we were able to collect data from a larger sample of participants. Finally, following best practices in behavioral research, we pre-registered the methods and analyses for this study (https://aspredicted.org/6fv8g.pdf).

Methods

Participants: Participants were 380 college students from the University of California, Riverside (180 females, 198 males, 2 non-binary) completing a series of studies in exchange for partial course credit. The average age of the participants was 21.3 (SD= 3.04, range 18 to 44). We were able to recruit more participants for this study than for Study 1 because the class that we drew participants from had higher enrollment this quarter.

Video: We used a 42-second long Five Star Notebooks advertisement found on YouTube that was originally posted on June 1, 2019. Our participants were college students and so using an ad for a company that makes school supplies made the ad more relevant. We also deliberately chose an ad that contained much more informational content so that participants would have more to remember.

Questionnaire: The survey was made on Qualtrics and was similar to the survey used in the first study. The main differences were the inclusion of a free recall memory task and changes to the multiple-choice quiz questions to reflect the new ad. The free recall memory task asked participants to list up to 10 things from the ad "that come to mind (product attributes, benefits, brand name, price, etc.)" with 10 blank text boxes. Participants were told they did not have to fill in

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Variables	*M*		*SD*		*t*-stat	*p*	Regression		
	1x (n = 195)	1.25x (n = 185)	1x	1.25x			*b*	*t*-stat	*p*
Enjoyment	5.14	4.81	1.33	1.51	-2.07	.02	-0.30	2.32	.04
Entertainment	4.94	4.56	1.48	1.46	-2.19	.01	-0.33	2.54	.02
Bad Experience	2.35	2.59	1.44	1.52	-1.59	.11	0.20	-1.59	.11
Informative	5.51	5.30	1.26	1.24	-1.69	.09	-0.19	1.69	.09
Attention	5.70	5.50	1.38	1.59	-0.85	.19	-0.13	1.31	.39
Understanding	6.35	6.06	0.85	1.12	-2.62	.005	-0.26	2.84	.01
Relaxing	4.15	3.63	1.55	1.53	-3.31	.001	-0.5	3.31	.001
Stress	2.55	2.83	1.53	1.66	1.58	.09	0.26	-1.70	.12
Ad Message Understanding	6.12	5.85	1.10	1.24	-2.25	.03	-0.25	2.25	.03
Ad Relevance	5.37	5.16	1.47	1.36	-1.46	.15	-0.14	1.46	.15
Ad Believable	5.72	5.44	1.11	1.11	-2.50	.01	-0.26	2.50	.01
Product Usage	5.37	5.08	1.35	1.46	-2.07	.04	-0.25	2.07	.04
Brand Sentiment	5.53	5.25	1.09	1.23	-2.00	.02	-0.22	2.40	.05
Perceived Speed	4.08	4.69	1.59	1.53	3.84	<.001	0.67	-3.84	<.001
Audio Distortion	2.93	3.45	1.51	1.75	3.08	.002	0.50	-3.08	.002
Video Distortion	2.35	2.70	1.23	1.38	2.65	.09	0.32	-2.65	.009
Quiz Score	3.35	3.28	0.97	0.99	-0.52	.53	-0.05	0.64	.60
Items Recalled	5.39	4.82	2.13	2.05	-2.39	.01	-0.51	2.64	.02

Table 3. Descriptive Statistics for Dependent Variables, regressions, and t-tests of Normal vs. Fast Groups

all the blanks.

Procedure: The participants first watched the video advertisement at normal or 1.25x speed, then answered questions about their experiences watching the advertisement on a 7-point Likert scale. Participants then completed the free-response recall memory task and the five-item multiple-choice memory quiz. Finally, we asked about participants' demographics, prior familiarity and usage of the brand, and typical behavior towards video advertisements outside this study.

Results

Similar to **Table 2**, **Table 3** shows descriptive statistics for all of the dependent variables we measured. We created scores for the memory tasks by simply counting the number of items recalled in the free recall task and by totaling up the number of correct responses out of five on the multiple-choice quiz. The group mean for the normal speed group was higher than the increased-speed group for all the dependent variables listed above except for stress, perceived speed, and audio/video distortion.

In addition to the *t*-tests in **Table 3**, we also ran linear regressions for the dependent variables as a function of speed while controlling for individual differences in age, gender, and prior brand familiarity and usage. We do not report the effects of the control variables since they are not the focus of our research.

Relative to the normal speeds, faster speed made for a less enjoyable (b = -0.30, t = -2.07, p = 0.039), less entertaining (b = -0.33, t = -2.19, p = 0.020), and less relaxing video (b = -0.490, t = 3.31, p =.001). Faster speeds also reduced self-reported understanding of the video (b = -0.261, t = -2.62, p = 0.009) and of the ad's message (b = -0.252, t = 2.25, p =0.025). This reduced understanding was supported by the finding that faster speeds reduced the number of facts recalled about the ad (b = -0.501, t = -2.39, p = 0.017), although there was no difference in performance on the multiple-choice quiz (b = -0.05, t = -0.52, p = 0.603). Perhaps more importantly, participants in the faster speed condition also found the ad to be less believable (b = -0.260, t = 2.50, p = 0.013), were less likely to want to use the product advertised (b = -0.247, t = 2.07, p = 0.039), and felt worse brand sentiments toward Five Star Notebooks (b = -0.22, t = -2.00, p = 0.047).

Regressions also found that faster speeds did not have a significant impact on participants' attention (b = -0.13, t = -0.85, p = 0.394), stress (b = 0.26, t = 1.58, p = 0.116), perceived ad relevance (b = -0.138, t = 1.46, p = 0.145) and informativeness (b = -0.186, t = 1.69, p = 0.093), or if the ad was a bad experience (b = 0.204, t = -1.59, p = 0.113). However, the direction of these effects is also consistent with a negative impact of faster speeds as well.

The negative impacts of faster speeds could be at least in part due to the fact that the 1.25x speed video seemed faster to participants (b = 0.669, t = -3.84, p < 0.001) and had significantly increased audio (b = 0.497, t = -3.08, p = 0.002) and video (b = 0.317, t = -2.65, p = 0.009) distortions. We did not anticipate such distortions since the way videos are sped up on YouTube usually does not impact audio/video fidelity on a modern device (especially for 1.25x speed). Since participants completed the study on their own devices, it is possible that they did not have sufficiently fast processors or internet speeds to accommodate the faster speed video.

We also analyzed participants' self-reported behaviors regarding their usual ad watching practices. In our college student sample, participants reported that they most frequently encountered advertisements on YouTube (75.7%), social media sites such as Facebook and Instagram (18.3%), and only a small portion on more traditional media on Hulu (2.4%) and television (1.8%). More importantly, only 16.8% of participants report watching ads when they are unskippable; instead, most participants do something else while the ad plays (72.8%) or even switch to a different app or video entirely (10.5%). This suggests that companies have an uphill battle to capture consumer attention during ads. Simply using skippable ads is not the answer; 93% of participants reported that they always skip ads and the remaining 7% sometimes do.

Discussion

Unlike for the Vrbo ad in Study 1, we found many significant effects of video speed for the Five Star Notebook ad. We hypothesized that speeding up the ad will increase people's attention towards the ad but that faster speeds will also reduce ad memory. While we did find the hypothesized negative effects on ad memory, we also found many other negative impacts of faster speeds on viewers' experience watching the ad and their brand sentiments. We also did not find our hypothesized positive effect on attention, although our attention measure was based on a single self-reported item.

GENERAL DISCUSSION

Two studies tested if speeding up playback would affect people's experience watching an non-skippable ad, as well as their brand sentiments and ad memory. The results were different from what we expected. We had thought that since people generally dislike video ads that play before and during their chosen video, speeding up the ad might make the experience more tolerable. The results show the exact opposite. Not only did the participants enjoy the increased speed ad significantly less than the normal speed ad, but they also understood it less. One potential explanation for results is that ads on YouTube and other social media/streaming platforms are much shorter than they are on live television. Most of the non-skippable ads on YouTube are no longer than thirty seconds and some are as short as five seconds. If the speed is increased for an ad that already is very short, then the ad may be over before viewers even realize what was being advertised. This can greatly decrease the effectiveness of these ads and the viewers' engagement.

Limitations

There are limitations to this study. This study was conducted with college students with an average age of 21.3 years and results could be different for older people, not only due to differences in patience but also because younger people may have grown less accustomed to watching

advertisements having grown up with YouTube instead of broadcast television. The video we chose was 42 seconds long and geared toward students, so it also does not accurately represent ad lengths and content for the general ad population. This study also focused on video ads likely to be seen on places like YouTube. Ads on live television are generally longer and so this study may not accurately represent live television ads.

FUTURE DIRECTIONS

Future studies should include older people from diverse backgrounds using ads relevant to their age range. Adults of various ages may have similar or entirely different reactions to sped-up ads than people in their twenties. In the age of data analytics, people are now shown more ads that are relevant to their activities on the internet, so different age groups will likely receive different ads. Given this, it is not too far-fetched to think different age groups may also respond differently to changes in playback speed. Future research could also focus viewers' responses to ads that play during live television. Ads of varying lengths should also be studied to see if people's responses to sped-up ads change with the length of ads. Another direction could be to ask participants to predict how they will react to a sped-up ad versus a normal sped ad before they watch the ad. Afterward, researchers could ask participants about their experience watching the ad, test them on retention of ad content, and ask them if they were correct about their prediction. Although our study found faster ads to be worse experiences, it is possible that people still choose to watch sped-up ads. Finally, using eye-tracking methods to objectively measure attention to the video would provide a more accurate study of if attention increases for faster video speeds as past studies suggest.

CONCLUSION

This study still needs replication and further research to confirm the generalizability of its findings, but its results found that speeding up ads did not improve people's enjoyment, retention, understanding, or brand sentiments. It did the opposite. The results of this study could help further the development of effective advertising by showing there is a limit to how fast ads can be shown to ensure ad effectiveness. It also points to a need for advertisers to find different methods of getting consumers' attention in the digital media age.

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Behavioral Effects of Administering CTEP Treatment in a Mouse Model of Fragile X Syndrome

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ABSTRACT

Fragile X Syndrome (FXS) is a genetic neurodevelopmental disorder that causes autism and intellectual disabilities: exhibiting hyperactivity, elevated anxiety, and impaired cognitive/sensory processing. These deficits result from mutations in the X-linked gene Fragile X messenger ribonucleoprotein 1 (*Fmr1*). *Fmr1*-knock-out (KO) mouse models have shown consistency with observations in humans, displaying seizures and sensory processing deficits. Utilizing *Fmr1*-KO mice to identify a potential treatment for these symptoms, we administered a drug called 2-chloro-4-((2,5-dimethyl-1-(4-trifluoromethoxy)phenyl)-1H-imidazole-4-yl) ethynyl)pyridine (CTEP) to KO mice and measured behavioral changes. CTEP inhibits metabotropic glutamate receptor pathways, which are upregulated in FXS. Two types of experiments were run: open field test (OFT) and elevated plus maze (EPM), commonly used to study anxiety and hyperactivity. Experimental mice with higher anxiety depict decreased exploration and more time spent near the arena's wall or closed arms. We found that CTEP reduces the distance traveled in the OFT across both wild-type (WT) and KO groups, suggesting reduced locomotion. There were no statistically significant differences in time spent in EPM closed arms between WT and KO mice, indicating no treatment of anxiety. These results suggest that more effective intervention is needed to target anxiety deficits related to FXS.

Keywords: Fragile X Syndrome, anxiety, hyperactivity, CTEP, mGluR5

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Diane Le

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Behavioral Effects of Administering CTEP Treatment in a Mouse Model of Fragile X Syndrome

INTRODUCTION

Fragile X Syndrome (FXS) is caused by a mutation in the Fragile X Messenger Ribonucleoprotein 1 (*Fmr1*) gene. Located on the X chromosome, it produces a protein called Fragile X Messenger Ribonucleoprotein (FMRP), which plays a crucial role in cell function[1].

Resulting in hyperactivity, impaired cognitive function, and sensory processing deficits[2], the mutation of *Fmr1* causes a loss of FMRP, resulting in increased levels of protein synthesis, influencing brain plasticity[3]. *Fmr1*-knockout (KO) mouse models have shown consistency with observations in humans, displaying symptoms like altered startle responses, high anxiety, and processing deficits. These similarities suggest that animal models of FXS may provide a secure foundation for understanding the underlying mechanisms of FXS and may facilitate new approaches to understanding the mechanisms of basic sensory processing behaviors in humans. In this study, we used the drug 2-**c**hloro-4-((2,5-dimethyl-1-(4-(**t**rifluoromethoxy)phenyl)-1H-imidazol-4-yl) **e**thynyl)**p**yridine (CTEP), a long-acting allosteric inhibitor of metabotropic glutamate receptor unit 5 (mGluR5)[10] to test whether chronic pharmacological mGluR5 inhibition reverses FXS phenotypes in a fully developed mouse brain[9]. In Fragile X Syndrome (FXS), mGluR5 is upregulated, altering brain circuitry and elevating hyperexcitability. The signaling of mGluR5 activates a phosphatidylinositol-calcium second messenger system, which may be involved in regulating neural network activity and synaptic plasticity[11]. Using CTEP, we are testing whether its treatment effects extend to behavioral assays as shown by Hamilton et al., (2016), whose findings suggest that CTEP or its analog Basimglutant may potentially be an effective treatment for patients with impaired sensory cognition. Our question is the following: how does CTEP treatment impact anxiety and hyperactivity-related behavioral phenotypes? We utilized *Fmr1*-KO mice to test whether CTEP alleviates hyperactivity and anxiety symptoms by measuring behavior changes and comparing these changes to wild-type (WT) mice.

Two types of behavioral experiments were run: open field test (OFT) and elevated plus maze (EPM), which are commonly used to study anxiety-related behaviors in rodents[5, 6, 7]. Experimental mice with lower rates of anxiety typically depict less exploration and higher thigmotaxis (i.e., near the walls of the arena) in the OFT or spend more time in the closed arms of the EPM. By analyzing behavioral assays of CTEP effects on *Fmr1* KO mice, we gain insight into the impact of direct treatment on behavioral deficits of hyperactivity and anxiety.

MATERIALS & METHODS

Ethics Statement. All experiments and animal care/use protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Riverside, and were carried out in accordance with the NIH "Guide for the Care and Use of Laboratory Animals."

Subjects. Mice were obtained by breeding wild-type male and *fmr1*$^{+/-}$ female C57/Bl6J mice. Pups were weaned at P21 and group-housed until experimentation. The study used C57/Bl6J male mice of the *fmr1*-KO line and wild-type littermates at postnatal age P50-P90. They were provided with food and water *ad libitum*.

Genotyping. A standard tail snip was performed on mice for genotyping. Mice were anesthetized with a short-acting anesthetic (e.g., isoflurane). Tail tip removal was performed using sharp, sterile scalpel blades or scissors. Approximately 0.1-0.5 cm of tissue was removed.

EXPERIMENTAL DESIGN

Open Field Test. An open field test (OFT) consisting of four activity chambers was used and conducted in a standard-lit room. Before testing, mice were habituated to the room for 1 hour. Each mouse was placed in an open-box arena (43 x 43 x 43 cm) and roamed for 10 minutes during testing and video recording. Using SMART Video Tracking Software from PanLab Apparatus, we analyzed subject behavior during

the first 5 minutes of arena exploration. Arena designs are digitally overlaid on the recorded arena from TopScanLite, a software used to track the movement of the mouse models. Two aspects of behavior were characterized: **(1)** Total distance covered (mm) during the timed portion of the experiment; **(2)** Thigmotaxis, or percent time that subjects remain adjacent to the outer wall (depiction of anxiety)[6].

Elevated Plus Maze. An elevated plus maze (EPM) consists of four perpendicular arenas with open and closed walls. Before testing, mice were habituated to the room for 1 hour. Each mouse was placed in the arena, roaming free for 10 minutes during testing while video recording took place. All data were manually recorded using a timer to start and stop when the mouse was in and out of the closed arms. Bouts were recorded each time a mouse entered the arena with the closed walls. Two aspects of the elevated plus maze were characterized using this protocol: **(1)** Total time spent in closed arms during the timed portion of the experiment; **(2)** How many times the mouse models entered and left the closed arenas.

Data Analysis. Statistical comparisons were run using GraphPad Prism, on which we ran: **1)** Two-way ANOVA to find the main effects and mixed effects of genotype, treatment, and condition. **2)** Student's t-test to compare two groups at a time. To determine statistically significant differences between groups/treatments, a two-way ANOVA was used with Tukey's *post hoc* test to correct multiple comparisons.

Drug Administration. Stock solutions of CTEP (MP Biomedicals) were prepared regularly and frozen until use, when they were thawed, vortexed, and then administered. All drugs and vehicle control solutions were administered daily for 10 days by a stainless steel curved oral gavage tip (Cadence Science, product #7910). Gavage tips were soaked in ethanol, rinsed with DI water, and autoclaved before each use. Mice received 2mg/kg CTEP per 48 hours for 10 days.

5-minute recordings of mouse behavior in the respective arenas were analyzed prior to 10-day CTEP treatment (precondition) and following treatment (post-condition).

RESULTS:

The main goal of this study was to determine if 10 days of daily CTEP treatment, compared to the vehicle, reversed phenological symptoms of hyperactivity and anxiety in an adult *Fmr1*-KO mouse model. Looking at OFT % time in the thigmotaxis and center, there was no difference between groups in time spent in each region for genotype and treatment. Running a two-way ANOVA test, mice generally spent more time in outer regions compared to inner regions. Studying distance traveled for OFT, the figures depict distance traveled over two main areas: thigmotaxis and center. No statistically significant data was seen between groups using a two-way ANOVA test. In EPM of % time in closed arms, there was statistically significant data between CTEP KO pretreatment (pre) and CTEP KO post-treatment (post) depicting that there was an increase from pre to post. A t-test was used to determine the differences between groups and treatments, and a Tukey's *post hoc* test was used to correct for multiple comparisons.

Behavioral Effects of Administering CTEP Treatment in a Mouse Model of Fragile X Syndrome

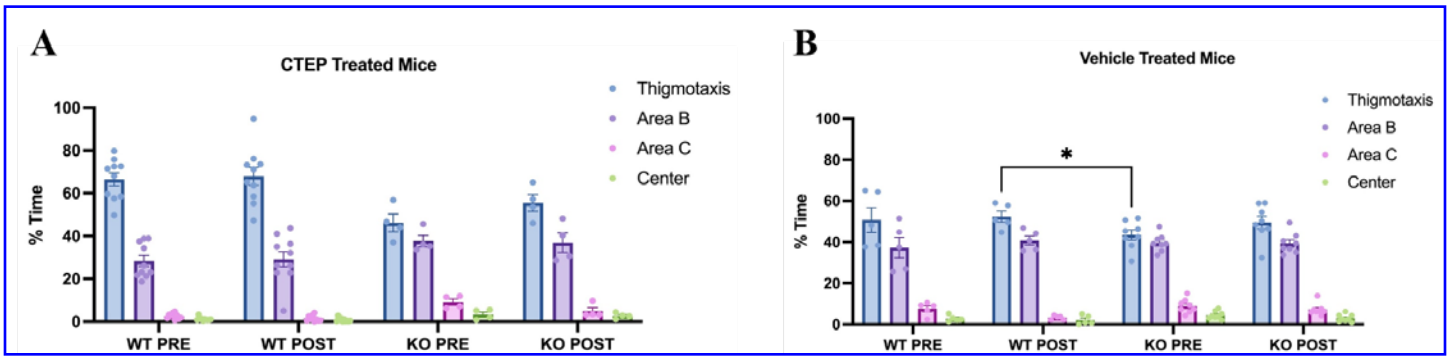

Figure 1. Graphs show the effects of CTEP and vehicle (Veh) treatment in pre- and post-wild type and knockout mouse models in the different arenas. **(A)** There was no difference between groups in how much time they spent in each region. Generally, mice spent more time in outer regions compared to inner regions. WT Pre: n=10, WT Post: n=10, KO Pre: n=4, KO Post: n=4. **(B)** The graph shows statistically significant differences between WT post-treatment with vehicle (control treatment) & KO pre-treatment. The thigmotaxis and Area B have the highest % of time spent. Area C and Center zone have the least % of time spent. WT Pre: n=5, WT Post: n=5, KO Pre: n=8, KO Post: n=8, * indicates $p \leq 0.05$. In both graphs, the y-axis depicts % time that the mice spent in each area. A two-way ANOVA was used to determine statistically significant differences between groups and treatment, and a Tukey's *post hoc* test was used to correct for multiple comparisons. In both graphs, the y-axis depicts % time that the mice spent in each area. A two-way ANOVA was used to determine statistically significant differences between groups and treatment, and a Tukey's *post hoc* test was used to correct for multiple comparisons.

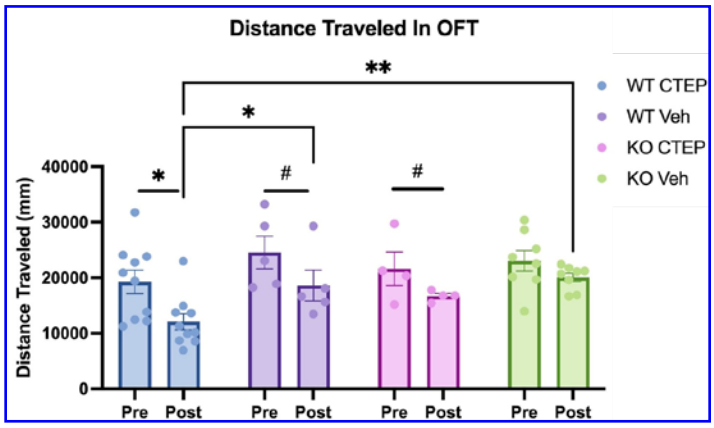

Figure 2. The y-axis depicts the total distance that mice traversed. The graph shows reduced distance traveled between pre and post in WT treated with CTEP (significant), WT treated with vehicle (trending), and KO treated with CTEP (trending). A two-way ANOVA was used to determine statistically significant differences between groups and treatment, and a Tukey's *post hoc* test was used to correct for multiple comparisons. WT CTEP: n=10, WT Veh: n=5, KO CTEP: n=4, KO Veh: n=8, * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, # indicates $p \leq .076$ (trending)

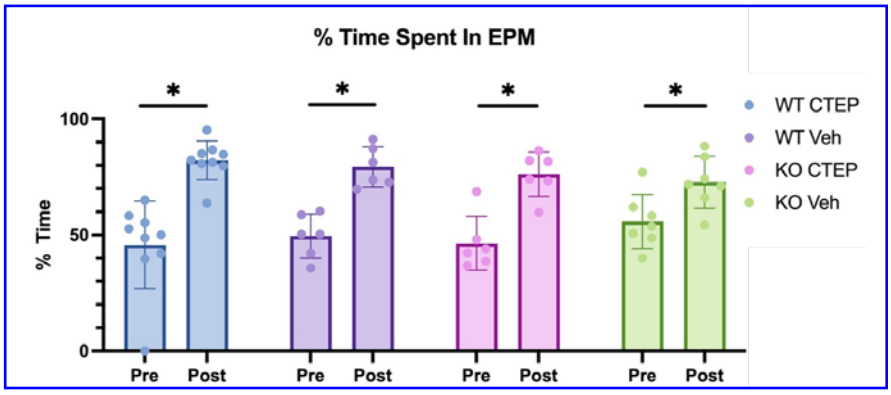

Figure 3. This graph shows % time spent in EPM in closed arms by comparing WT and KO CTEP and Veh mouse models. The y-axis depicts % time spent in the closed arms of the EPM. Generally, mice spent more time in the closed arms in the post-condition compared to pre condition, regardless of genotype and treatment type. A two-way ANOVA was used to determine statistically significant differences between groups and treatment, and a Tukey's *post hoc* test was used to correct for multiple comparisons. WT CTEP: n=9, WT Veh: n=6, KO CTEP: n=6, KO Veh: n=7, * indicates $p \leq 0.05$

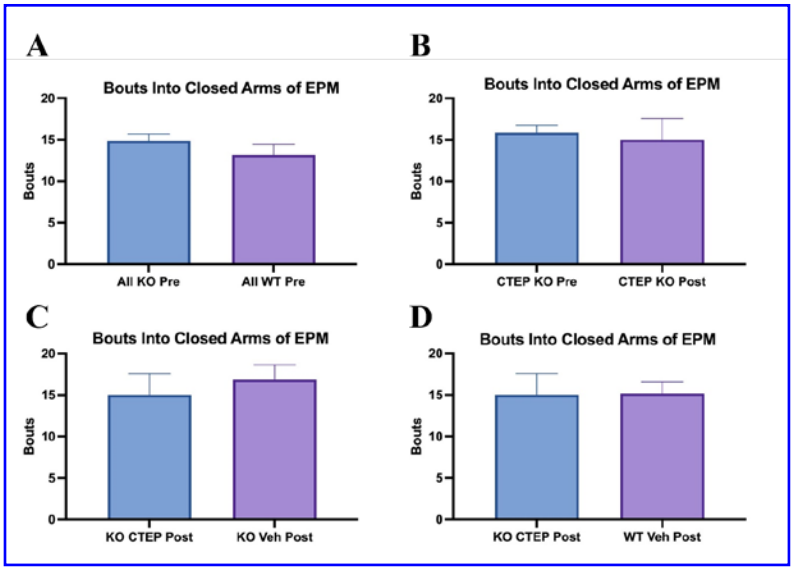

Figure 4. The graphs shows the number of times the mouse models entered the closed arms in EPM (bouts), comparing it to the different combinations of genotypes. **A)** All WT Pre: n=15, All KO Pre: n=12. **B)** CTEP KO Pre: n=4, CTEP KO Post: n=4. **C)** CTEP KO Post: n=4, Veh KO Post: n=8. **D)** CTEP KO Post: n=4, Veh WT Post: 5 Among the graphs, there were no statistically significant differences between groups in how much time they spent in each region. Generally, mice spent more time in outer regions compared to inner regions. The y-axis depicts the bouts that the mice spent in each area. A t-test was used to determine statistically significant differences between groups and treatments, and a Tukey's *post hoc* test was used to correct for multiple comparisons.

DISCUSSION

By using the mouse model of FXS, the *Fmr1* WT and KO mouse models are used to assess if behavioral symptoms of FXS have been reduced through the treatment of CTEP. Administering CTEP to *Fmr1*-KO and WT mice affected their behavior in OFT and EPM in the following ways: **1.** Both genotypes reduced distance traveled following treatment with CTEP (**Figure 2**), **2.** Only WT showed a reduction in distance traveled with Veh (**Figure 2**), **3.** KO that received the vehicle did not change the distance traveled with OFT (**Figure 2**), **4.** Between WT & KO mice and between CTEP treatment and vehicle, there were no significant differences in % of time spent in closed arms of EPM (**Figure 3**).

Studying the effects of 10-day CTEP treatment, locomotion in both genotypes was reduced, but there are no physical signs that hyperactivity and anxiety were decreased. Treatment was not improved significantly in alleviating these symptoms of hyperactivity and anxiety between CTEP and Veh mouse models. The behavioral assays of WT and KO may not have been sensitive enough to detect the differences in genotype because the WT mouse models used were glutamates, born in the same cage as the KO mouse models. Environmentally, WT models are influenced by KO, which is a huge factor in how the experiment is affected. With no statistically significant differences in % time spent in closed arms following CTEP treatment compared to vehicle, there was no indication of treatment of anxiety. Reducing hyperactivity, CTEP was shown to reduce the distance traveled in OFT of both WT and KO mouse models, suggesting reduced locomotion but no alleviation of KO-specific deficits.

Given that the mouse models were treated by oral gavage, this may have contributed to elevated anxiety in the subjects of the study. A future step that will address this is to administer treatment through water consumed regularly by mice to limit anxiety given by oral gavage. CTEP did not alleviate knockout-specific hyperactivity in this study; we suspect tolerance to CTEP may have been acquired[7]. They have shown that tolerance is built through continual CTEP treatment, as seen in clinical studies that show a lack of improvement of symptoms in humans with FXS and in rodent models. In the future, a single acute dose of CTEP will be provided instead of a chronic 10-day treatment. Using mouse models, we will assign each mouse a single dose at a younger age (~P28) and test their behavioral phenotype when they are older (~P60). As shown by Lovelace et al. (2020), there was an improvement in the *Fmr1*-KO mouse model of Fragile X Syndrome through treatment using minocycline. Administering the minocycline using a similar 10-day treatment protocol as that used in this study,

led to improvements as shown in electroencephalogram (EEG) measures[12]. Continuing this experiment, we will use EEG biomarkers to identify potential electrophysiological improvements following acute CTEP treatment. It is vital to continue searching for effective treatments for humans with FXS to decrease sensory symptoms of FXS and to further understand the underlying mechanisms of this condition.

CLINICAL RELEVANCE

A consistent symptom of FXS, sensory and auditory hypersensitivity is a prominent symptom noted in clinical and parent reports. Leading to increased anxiety and delayed language development, early childhood sensory processing abnormalities can lead to further disruptions in development. Taken together with studies that have shown alleviation in symptoms of mouse models with FXS through the treatment of CTEP, these studies point to mGlu5 inhibition as a therapeutic avenue for humans with FXS[13].

In neurodevelopmental disorders research and drug development, correlations have been made with the treatment of CTEP to suggest that it effectively reduces behavioral symptoms of sociability deficits, increased anxiety, hyperactivity, and sensory hyperexcitability[15]. CTEP has been tested as a potential therapeutic in the neurodevelopmental disorder: Fragile X Syndrome. A further evaluation of how CTEP affects a broad range of phenotypes displayed in mouse models is required before designing outcome measures for humans.

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Patriot Pirates? A Reassessment of American Privateers in the Revolutionary War

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Alejandra Dubcovsky, *Ph.D., Department of History*

ABSTRACT

All major combatants of the American Revolutionary War (1776-1783) deployed privateers to disrupt shipping in the Atlantic Ocean. American privateers uniquely experienced the war as they broke the British blockade of North America, raided British territory, and disrupted international trade. American privateers are often depicted as legalized pirates or profiteers. However, the reality of privateering is much more complex. This article analyzes primary sources, including memoirs, correspondence, and secondary historiographical texts to explore how privateers have been misrepresented in history. These sources reveal a legally and personally complicated affair that does not align with existing understandings of the topic. This article posits that previous understandings of privateering, featured in select primary and secondary sources, are inaccurate and deny historians valuable information. This article seeks to recover privateers' place in the broader history of the American Revolution and allow for an integration of their experiences in future historiography by uncovering their muddled history.

KEYWORDS: American Revolution, Continental Navy, marque, privateer, piracy

Joaquin Lopez

Joaquin Lopez is a fourth-year history major. He studies the lives and historiography of American privateers during the American Revolution under Dr. Alejandra Dubcovsky's guidance. Joaquin presented his research at the UC Riverside Undergraduate Research & Creative Activities Symposium, and his work was selected for publication in the UCR Department of History's Cornerstone journal. After graduating, Joaquin will pursue an M.A. in history and continue his research on Atlantic history.

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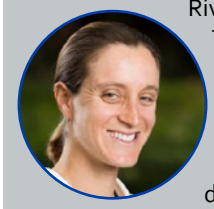

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Patriot Pirates? A Reassessment of American Privateers in the Revolutionary War

INTRODUCTION

Spurred by scenes of "exercising [...] soldiers, […] the sound of martial music and the call for volunteers," fourteen-year-old Andrew Sherburne hurried to enlist with the American forces during the early years of the American Revolution. Following his eldest brother's footsteps, he enlisted with the Continental Navy. After two years aboard the Continental Navy sloop *Ranger,* he returned home in 1780 and discovered that his older brother and father had died. Unable to find reliable employment, Sherburne found himself without the means to provide for his mother and sisters.[1] He agreed to temporarily serve aboard a privateer ship, *Greyhound*, to make money before rejoining the Navy. Unfortunately, British privateers captured the *Greyhound* and its crew. Sherburne would be held as a pirate and traitor for the remainder of the war. For three years, he endured severe malnutrition at Mill Prison and prison ship *Jersey*.[2] Sherburne returned home in 1783 penniless and permanently handicapped.[3] His memoir, published in 1831, provides a harrowing account that encompasses the experiences of thousands of other privateers. It does not detail famous battles or the experiences of officers and politicians. Sherburne's memoir, at its core, is a "ground-up" view of how the general population experienced the war. However, the story of Andrew Sherburne and his fellow privateers is often forgotten or misrepresented in historical discussions.

During the American Revolutionary War, the United States, France, and Britain employed thousands of privateers on ships like *Greyhound* to disrupt merchant shipping in the Atlantic Ocean. Privateers were civilians that held special government commissions to engage in naval warfare. Historians have struggled to represent the experiences of these sailors, depicting them as a vital supplement to the Continental Navy but also closely tied with piracy, greed, and unpatriotic behavior. An analysis of surviving memoirs, correspondence, letters of marque, and ordinances uncovers American privateers' complex and muddled history during the war. These sources reveal that privateering was a legally complicated affair undertaken by individuals whose behaviors and motivations are difficult to homogenize. A reassessment of privateers thus helps resituate privateers within the history of the American Revolution and focus on the broad history of the war through the experiences of ordinary people. Privateers allow us to reexamine why and how people participated in the American Revolution, explore legal frameworks developed by the United States to deal with its constituents, and how its citizens interacted with the emerging nation-state.

HISTORICAL AND HISTORIOGRAPHICAL PERSPECTIVES ON PRIVATEERING

Given the nature of their occupation, privateers were subject to criticism from various groups. For instance, Continental Navy and Army officers were critical of privateers' behaviors and effects on the war. Among members of the Continental Navy, Captain John Paul Jones was the most fervent critic of privateering. Jones protested that he had to sail alongside self-interested men who "pretend to love their country."[4] Jones' principal concern was that privateers were siphoning human resources from the Navy by offering higher wages or employing deserters.[5] In a letter to Robert Morris, Jones concludes that "sordid adventurers in privateers [sport] away

1 Robert H. Patton, *Patriot Pirates: The Privateer War for Freedom and Fortune in the American Revolution* (New York: Pantheon Books, 2008), 168-169.

2 Andrew Sherburne. *Memoirs of Andrew Sherburn, a Pensioner of the Navy of the Revolution* (Providence: H.H. Brown, 1831), 35-36.

3 Sherburne, 126-127.

4 Kylie A. Hulbert, *The Untold War at Sea: America's Revolutionary Privateers* (Athens: University of Georgia Press, 2022), 159.

5 See also C. Kevin Marshall, "Putting Privateers in Their Place: The Applicability of the Marque and Reprisal Clause to Undeclared Wars," *The University of Chicago Law Review* 64 (1997), 970. The failure of the 1779 Penobscot Expedition also created animosity between priva-

the sinews of our marine."[6] Esek Hopkins, commander-in-chief of the Continental Navy, echoed Jones' criticisms of privateering. Hopkins, in one letter, claimed that a third of the men assigned to Navy ships had joined privateers, leaving the Navy with a severe manpower shortage.[7] Hopkins would later defend Jones in an incident where Jones boarded a ship and took four privateers into the Navy's service as he suspected they were deserters.[8] Finally, George Washington expressed his disapproval of privateers, characterizing them as "inconsistent and disloyal" and entirely self-interested.[9] These primary sources depict privateers as a nuisance to the Continental Navy and as unpatriotic. Historians like Gardner W. Allen and James M. Volo have reiterated the criticisms levied against privateers. In the conclusion of Allen's 1913 book *Naval History of the American Revolutio*n, he states that if one-half of the "men, money, and energy absorbed in privateering" had been invested into the Continental Navy, then it "would have provided a force able to act offensively against the British navy to some purpose."[10] Volo's *Blue Water Patriots* asserts that privateers enlisted because of "simple economic self-interest" and created significant manpower shortages in the Navy.[11] Jones, Hopkins, Washington, and Allen hold unpatriotic privateers responsible for the Continental Navy's shortcomings.

Other criticisms of privateering contributed to an association between pirates and privateers. In a 1789 article published in the *Gazette of the United States*, Benjamin Franklin declared the practice a "remnant of the ancient piracy" and called for its abolishment. In his article, Franklin accused privateers of being pirates with government protections, "wantonly and unfeelingly" destroying families, and continuing that destruction in post-war America.[12] Officers of the British Navy, who took hundreds of privateers as prisoners, also did not differentiate between pirates and privateers. The letters of marque did not guarantee a privateer's legal immunity from piracy charges. When captured by the British, privateers were "held under a bill of attainder charging them with both piracy and treason."[13] Privateers would be subject to poor conditions in prison ships like *Jersey* and many would die. Privateers were also excluded from prison exchanges by Britain, being a "foe unworthy and undeserving of such consideration."[14] Privateers were not differentiated from pirates by the British and Americans.

Historiographical discussions have also deepened privateers' ties to piracy. Robert H. Patton's 2008 book, *Patriot Pirates*, claims to be a history of privateering during the American Revolution. However, the book's title only indicates that the negative perception of privateers persisted into the twenty-first century. The book's flap copy and introduction describe American privateers as part of a "massive seaborne insurgency involving thousands of money-mad patriots plundering Britain's maritime trade."[15] The terminology used

teers and the Continental Navy.

6 Hulbert, 159-169.

7 Hulbert, 151. See also *Thomas Butts to his cousin*. Butts, a British sailor captured by privateers, states that only "seasick country bumpkins" are available to the Continental Navy.

8 Hulbert, 151.

9 Hulbert, 159.

10 Gardner Weld Allen, *A Naval History of the American Revolution* (New York: Russell & Russell, 1962), 663.

11 James M. Volo, *Blue Water Patriots: The American Revolution Afloat* (Connecticut: Praeger Publishers, 2007), 45.

12 Benjamin Franklin, "Against Privateering."

13 Volo, 45.

14 Hulbert, 66. See also Patton, 33-34. The 1777 Treason Act denied due process for privateers and only allowed them their freedom if they chose to serve in the British Royal Navy.

15 Patton, flap copy text.

in this synopsis portrays privateers as an unsanctioned group of sailors, like pirates, greedily hunting down merchant ships. A more objective perception would acknowledge the gray area privateers occupied during the war. Some privateers committed illegal captures, employed deserters, and offered higher wages to draw sailors away from the Continental Navy.[16] Other privateers were praised for their contribution to the war effort and demonstrated alternative perspectives not found in oversimplified descriptions of privateers.

Individuals like John Adams and Nathanael Greene were in favor of privateering. While both John Adams and John Paul Jones advocated for a larger Continental Navy, Adams conceded that privateers were an effective way to bolster the nation's naval fighting capabilities.[17] Bureaucratic issues delayed the organization of the Continental Navy in its early years. Conversely, there was an established precedent for using privateers in wartime, such as during King George's War. State governments could hire privateers in such a way that allowed hundreds of ships to sail out. In a letter to Pennsylvania delegate Benjamin Rush, Adams wrote that "there should not be the least obstruction to privateering [...] I firmly believe that one sailor will do us more good than two soldiers."[18] While Benjamin Franklin denounced them after the war, he assisted privateers operating from France. Franklin also organized a strategy that intensified attacks on "British shipping […] in order to fan the fire of public resentment against the further prosecution of the war."[19] Privateers were a persistent threat to the British economy throughout the war, exacerbating war weariness in England. Like Adams and Franklin, Nathanael Greene saw their potential and financed several privateers throughout the war. Greene never explicitly stated any criticisms about privateers or their behaviors but did believe that they were a vital extension of the nation's formal armed forces.[20] Opinions on privateering among ordinary American people seemed to vary; they were seen as the "lifeblood of New England" as they brought in goods and supplies that were otherwise scarce. However, they were also periodically blamed by the public for the limited availability and high prices of goods.[21] Although these perspectives of privateering by other figures of the American Revolution do not address the criticisms laid out by Allen, Franklin, or Jones, they indicate that perspectives on privateering were contested before the American Revolution was over.

THE LEGAL AND PERSONAL COMPLEXITIES OF PRIVATEERING

To better understand privateering's nature, it is crucial to recognize that privateers, in contrast to pirates, had to operate under strict regulations and oversight. An overview of relevant ordinances and law demonstrates how they were regulated and organized. Instructions published in April 1776, for example, outlined rules for all privateers serving under the Continental Congress' letters of marque.[22] These rules specified whom privateers could target, that being any ship transporting "soldiers, arms, gunpowder, ammunition, provisions or any other contraband goods, to

16 *See Thomas Butts to his cousin.* Butts recalls his capture by privateers disguised as a friendly British vessel.

17 See Allen, *A Naval History of the American Revolution*, 662-664. Although Gardner Allen argues that a stronger navy would be more effective, he recognizes that privateers emerged out of necessity.

18 Hulbert, 141.

19 Volo, 224.

20 Patton, 107-108.

21 Marshall, 964. See also Marshall, 966-968. Preexisting moral criticisms from before the American Revolution were also a source of the public's animosity towards privateers.

22 The same instructions published in 1776 had been in effect in Massachusetts since late 1775 through the *Act For Encouraging The Fixing Out Of Armed Vessels*.

any of the British Armies or Ships [...] employed against the colonies."[23] The document also instructed privateers that they had an obligation to report any captures to an admiralty court immediately and ensure the humane treatment of all prisoners. Finally, the instructions warned that any privateers found breaking the rules would have their letter of marque revoked, bond forfeited, and forced to pay reparations.[24] These rules emphasized the lawful seizure of goods and dissuaded privateers from recklessly attacking merchant shipping or causing unnecessary damage.

Several amendments were made to the initial ruleset throughout the war to deter overzealous privateers from illegally capturing ships. A 1781 ordinance published by the Continental Congress, for instance, clarified the pretenses under which a privateer could condemn a vessel and reiterated a privateer's duty to bring all prizes back to an admiralty court.[25] Another ordinance, published in 1782, resolved the "variance in the decisions of several maritime courts" regarding prizes by clarifying who was entitled to receive payment in the event of a successful capture.[26]

Privateers in service to the Continental Congress were under strict regulation and liable to lose their commissions and lawsuits if they violated the rules. Barzilla Smith and Gustavus Conyngham are examples of privateers punished for breaking these rules. John Hancock issued a $5,000 bond and a letter of marque to Smith in October 1776.[27] A letter from September 1777 indicates that Hancock revoked Smith's bond as a result of illegal capture and signed it over to the owners of the captured vessel.[28] Similarly, French authorities detained Gustavus Conyngham when he brought British ships to Dunkirk.[29] Like all American privateers, Conyngham and Smith had little time to make decisions and were isolated from Congress and the admiralty courts. However, these types of events were rare.[30] In addition, cases of illegal captures were not always purposeful and sometimes were a result of intentional deceit by merchants attempting to evade capture by disposing of paperwork.[31] Privateers, in contrast to pirates, were tightly restricted by law and had unique considerations that carried serious legal and personal risks.

People from all social classes signed up to become privateers without discussing their motivations. While there were cases of enslaved men put into service in privateers, most privateers were volunteers. Christopher Vail left a detailed account of his life during the war. Yet, the reasons why he became a privateer remain elusive. Vail enlisted in several units of the Continental Army and privateer vessels throughout the war. He was imprisoned twice by the British and held in deplorable conditions. Like Andrew Sherburne, Vail joined new privateer ships after escaping prison. In discussing Vail's journal, John O. Sands notes that Vail never gives "evidence of strong political opinions nor an awareness of the issues over which the war was fought."[32] However, Vail had some eagerness to fight as he served several tours with the Army and privateers throughout the war despite experiencing the conditions of British prisons and impressment. Nathaniel

23 *Instructions to the commanders of private ships or vessels of war*, II.

24 Instructions, III-VII.

25 *An Ordinance, Relative to the Capture and Condemnation of Prizes*.

26 *An Ordinance, for Amending the Ordinance, Ascertaining What Captures on Water Shall Be Lawfu*l.

27 *Privateer bond of Barzilla Smith, Joseph Chapman, and Elijah F. Payne*.

28 *Assignment of privateer's bond by John Hancock to Joseph Hewes and Robert Smith*.

29 Hulbert, 68-69.

30 Marshall, 971 citing Gardner Weld Allen, *Massachusetts Privateers of the Revolution* (Massachusetts: Massachusetts Historical Society, 1927), 15.

31 Hulbert, 68-69.

32 John O. Sands, "Christopher Vail, Soldier and Seaman in the American Revolution," *Winterthur Portfolio* 11 (1976), 54.

Patriot Pirates? A Reassessment of American Privateers in the Revolutionary War

Fanning's story is like Vail's. He served as an officer in the Continental Navy, working under John Paul Jones before leaving to take charge of a privateer, having "found Jones so insufferable that he politely refused any [...] place among his officers"[33] He also endured poor conditions under British capture. Nonetheless, he enlisted with another privateer after his release. It seems that some men joined privateers because they wanted to fight.

Unlike Vail or Fanning, Nathanael Greene became involved in privateering through the financing of the purchase of ships, arms, and provisions. Greene invested significant parts of his pay into funding privateers, reasoning that his "business" as a Continental Army officer required the materials supplied by privateers' captures. His investment was not for financial gain but to "annoy the enemy and consequently favor our cause."[34] While Greene's motivations are not explicitly patriotic, it was strategic with military matters prompting his involvement.

Promises of payment and wages made joining a privateer more attractive than the Continental Navy and allured many men. Men, like John Whiting, explicitly stated that they would only serve until they received payment.[35] Andrew Sherburne joined to support his widowed mother.[36] The promise of capturing a ship and cashing out a large prize was an attractive incentive to those who enlisted. However, privateers also knew that financial gain was not guaranteed as admiralty courts often prevented them from profiting in their venture. Admiralty courts forced privateers to prove that their captures were legal in cases that could take years to settle. Congress only established a formal court of appeals in May 1780, five years into the war. For most of the war, a privateer's right to appeal their case was not guaranteed in some states, and attempting to appeal a case carried the risk of exorbitant legal fees.[37]

Thomas Rutenbourgh spent a year in court trying to prove that they had conducted a legal capture as the captured ship's crew had thrown all identifying paperwork overboard. Rutenbourgh would lose the case and his prize and try to appeal. The courts denied Rutenbourgh's appeal and forced him to pay legal fees to Congress, resulting in lost funds for capturing an enemy ship.[38] Hugh Hill also had his prize confiscated by the Continental Congress and spent $1,056 in legal fees.[39] Gustavus Conyngham, mentioned previously, appealed to Congress for back pay owed him from prizes he had captured. The courts and commissioners would dismiss the appeal eighteen years after Conyngham's request.[40] These cases also influenced public opinion. In her discussion of the admiralty courts, Kylie Hulbert suggests that their legal battles left privateers in an "unpatriotic position" as they "came to be viewed as profiteers" despite acting on the Continental Congress' orders.[41] As a result of complex and constantly changing legal systems established by Congress, the privateering business could not guarantee financial gain. Even if Nathanael Greene did have underlying motivations based on the profit potential, the fact that he was bankrupt by the end of the war only further indicates that privateering was

33 Volo, 223.

34 Patton, 108.

35 Hulbert 30.

36 See also Sherburne, 35. Sherburne describes children "not a dozen years old" aboard *Greyhound*. It is not clear why they had joined the privateer ship.

37 Hulbert, 114.

38 Hulbert, 122.

39 Hulbert, 1-2.

40 Patton, 183-184.

41 Hulbert, 135.

not a completely profitable venture.[42] The admiralty courts prevented privateers from making a profit and unintentionally made privateers appear to be overly preoccupied with money.

Within the crews of the thousands of privateer ships that sailed during the war, there may have been several men who were the unpatriotic, self-interested pirates and deserters that Jones or Washington believed privateers to be. However, the notion that money solely motivated all privateers is invalid, as privateers knew admiralty courts were challenging to navigate. Many privateers adhered to the rules established by the Continental Congress even as Congress periodically placed embargoes on them and forced them to give up potential prizes. There was no uniformity in what motivated men to enlist as privateers. As a collective, privateers stand in a gray area where they can neither be classified as uncontrolled pirates nor hard-line patriots.

The captains, crews, and financiers of the two thousand privateer ships commissioned by the United States during the American Revolution represent a significant component of the American Revolution that has been subject to inaccurate characterizations that do not consider their unique circumstances. Understanding that privateering was legally complex and that privateers were not homogenous in their behaviors allows for a more productive discussion of privateers and their role in history. Some contemporary histories have demonstrated a new trend in the interpretation and integration of privateers into the popular history of the American Revolution. Kylie A. Hulbert's *The Untold War at Sea* discusses privateering from the privateers' perspective and explores the legal challenges they encountered. Nathan Perl-Rosenthal's *Citizen Sailors* does not exclusively explore privateering. However, he depicts privateers as a racially and ethnically diverse group that prototyped American citizenship. While privateers still stand in a gray area regarding their motivations and behaviors, historiography has trended toward a discussion that acknowledges and discusses the complexities of privateering.

CONCLUSION AND CONSIDERATIONS FOR FUTURE RESEARCH

By comparing the characterization of American privateers presented by individuals like John Paul Jones or Gardner Weld Allen and the information offered by memoirs, legal documents, and accounts, it is evident that privateers are misrepresented in American history. As with many other groups that participated in the war, American privateers were a diverse group whose motives and behaviors are difficult to generalize. Documents and historiographical accounts place privateers in an unclear position that is distinct from pirates and navy sailors but integral to the history of the American Revolution.

In the conclusion of her book, Kylie Hulbert suggests that a nuanced discussion of privateers can provide a "new thread into the complex story of the American Revolution [and] of the American experience."[43] Whereas the exploits of the Continental Army and Navy are well known, Hulbert believes that privateers were excluded because their "actions and experiences were unfamiliar and unique unto themselves"[44] By publicizing their experiences and making previously unfamiliar actions clear, privateers can enter the mainstream history of the American Revolution. Addressing the misconceptions surrounding privateering and understanding their conditions allows them to exist as more than a historical oddity or "legal pirates." Historians can then recognize privateers for their uniqueness and their potential historical value.

My research and that of other historians will allow privateers

42 Patton, 211.

43 Hulbert, 181.

44 Hulbert, 5.

to be better represented in future historiography of the American Revolutionary War. For example, historians of the "New Social History" school and the "Neo-Progressive" movement, as defined by Michael D. Hattem, would gain a valuable source of experiences by looking at privateers. The New Social History originated in the 1970s focuses on the lives of everyday people. This historiographical movement studies "history from the bottom up" as opposed to "Great Man" history. The Neo-Progressive movement, similarly, deals with the experiences of individuals. This movement highlights people's involvement in political and social change, "thereby integrating them into the larger political narrative of the Revolution."[45] Historians belonging to either of these historiographical movements can integrate the stories and experiences of privateers as they are better understood and removed from previous misrepresentations that sullied their standing in history.

Furthermore, Kylie Hulbert suggests that a better understanding of privateers will allow for their introductions into works that follow the "current trend that posits the war in its global context."[46] The "Atlantic" or "Neo-Imperial" schools of interpretation analyze the war beyond the North American continent and discuss the global consequences of the war.[47] Nathan Perl-Rosenthal's book fits into this school and represents a recent integration of privateers into a broader history. Perl-Rosenthal discusses privateers' relationship with concepts of citizenship and the Atlantic world. Privateers were some of the few Americans that went beyond the shores of North America, making them a valuable resource for historians of this movement.

Research into privateers still needs to be completed. Robert Patton asserts that "no study of Revolutionary privateering could pretend to give a complete picture of that complex era."[48] However, additional research into primary sources like memoirs and correspondence may help create a more accurate understanding of privateering and its role in the Revolutionary War. Kylie Hulbert's book represents a recent discussion of privateering that considers privateers' unique conditions. She compiled several anecdotes and stories of their everyday experiences to present a more definitive picture of privateering. Hulbert's book and sources present historians with a base that future discussions can expand or integrate into broader histories.

In the future, my research will examine how the masses viewed privateers. In this article, I focused much of my attention on how a few notable politicians and military officers described privateers. Examining how ordinary people perceived privateers would be conducive to a better and broader understanding of how privateers were perceived. This paper did not discuss some historiographical accounts that should be included in future analyses. Donald Shomette's Privateers of the Revolution, Eric Dolin's Rebels at Sea, and Edgar Maclay's History of American Privateers are notable texts that discuss privateers but are not accounted for in this article. Furthermore, research into privateering in other eighteenth-century conflicts, like King George's War or the Seven Years' War, would also help explain preexisting animosity against privateers that Kevin Marshall briefly mentions in his article. Although further research is necessary, privateers are now becoming more visible in history than ever before.

45 Michael D. Hattem, "The Historiography of the American Revolution," 2017, https://cdn.knightlab.com/libs/timeline3/latest/embed/index.html?source=19P0MD9TrV5Tx62DC3fImj_uNLA5lAsnV6TmRu2fWdL4.

46 Hulbert, 181.

47 Hattem.

48 Patton, XXI.

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Does Children's Negative Emotion Differentiation Relate to Emotion Regulation and Psychopathology?

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ABSTRACT

Negative emotion differentiation (NED) refers to experiencing negative emotions as being different from each other (e.g., sadness vs. fear). Prior literature has linked emotion regulation (ER) to internalizing psychopathology. The current study investigates how NED among pre-adolescent youth may moderate this relationship. Participants include 106 youth ranging from ages 7 to 11 (55.6% male, Mage = 9.3 years). NED scores were computed as the variance in self-reports of negative emotions across multiple time points. ER processes were measured using autobiographical emotion interviews and resting respiratory sinus arrhythmia (RSA) during a baseline task. Children's anxiety and depression symptoms were evaluated using validated measures. NED is expected to (1) relate to depressive symptoms, (2) moderate the effectiveness and selection of both adaptive and maladaptive ER strategies, and (3) moderate the relationship between RSA and psychopathology. Initial correlational and independent t-test analyses revealed that NED was not directly associated with psychopathology. Instead, NED was positively related to separation anxiety for youth who did not use support-seeking strategies. NED was also higher for children who did not use cognitive reframing strategies compared to children who did. Additional analyses revealed that resting RSA had a protective effect against some facets of psychopathology based on specific regulation strategies used. These findings have implications for interventions (e.g., mindfulness) that can improve mental health outcomes for youth.

KEYWORDS: Emotion differentiation, emotion regulation, child psychopathology, respiratory sinus arrhythmia (RSA)

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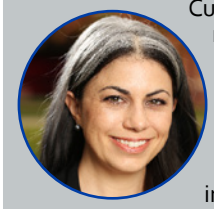

Dr. Davis is an Associate Professor in the departments of Psychology and Media & Cultural Studies at UCR. Her research focuses on understanding how emotion regulation relates to adaptive outcomes (e.g., learning) and maladaptive outcomes (e.g., anxiety) in childhood. Her goal is to identify regulatory strategies that children can use to effectively alleviate negative emotion and to identify individual differences in children's biology and social experiences that determine if they can regulate emotion effectively. She oversees the Emotion Regulation Lab which seeks to provide an empirical basis for interventions aimed at improving children's emotion regulation abilities and mitigating risk for maladaptive outcomes.

Gerardo Michel

Gerardo Michel is a fourth year Psychology major with a Philosophy minor. Over the past year, he has been working with Dr. Elizabeth L. Davis investigating what emotional processes may affect emotion regulation, and consequently, symptoms of depression and anxiety. He is involved in Dr. Davis' Emotion Regulation Lab, Dr. Tuppett Yates' Adversity & Adaptation Lab, and Psi Chi (the International Honor Society in Psychology). Gerardo plans to pursue a Ph.D. in Clinical Psychology to continue this research, conduct psychotherapy, and help address the youth mental health crisis.

Does Children's Negative Emotion Differentiation Relate to Emotion Regulation and Psychopathology?

INTRODUCTION

Background

The United States is experiencing a youth mental health crisis. According to the National Alliance on Mental Illness (2022), one in six youth aged 6 to 17 years in the United States experience a mental health condition each year. There are several ways in which this crisis can be targeted. One of these paths is through the process of emotion regulation which refers to how we respond to and modify our emotional processes to achieve a certain goal (Thompson, 1994). Previous research has found that emotional regulation is related to internalizing psychopathology while maladaptive strategy selection (e.g., avoidance and suppression) is more related to symptoms (Cavicchioli et al., 2022; Compas et al., 2017; Vanderlind et al., 2022; Werner et al., 2011) compared to more adaptive strategy use (e.g., cognitive reframing and support seeking).

There are many factors that could contribute to the relationship between emotion regulation and psychopathology. One possible factor is emotion differentiation, which broadly refers to how an individual experiences emotion as being different from each other (for example, distinguishing between sadness and fear; Barrett et al., 2001). Negative emotion differentiation (NED), which refers to emotion differentiation exclusively among different negative emotions, has been found to be related to internalizing psychopathology (Demiralp et al., 2012; Erbas et al., 2014; Kashdan & Farmer, 2014; Matt et al., 2016; Starr et al., 2017). NED has also been found to relate to emotion regulation, specifically when it comes to strategy use (Barrett et al., 2001; Brown et al., 2021) and strategy effectiveness (Kalokerinos et al., 2019). It is possible that emotion regulation, NED, and internalizing psychopathology (e.g., depression and anxiety) all relate to each other, which presents possible routes for clinical research. NED could serve to moderate the relationship between emotion regulation and psychopathology. Higher NED may be related to increased use of adaptive strategies, and thus, result in less symptoms of psychopathology.

Emotion regulation has been associated with psychopathology. One meta-analysis found that maladaptive strategy use such as avoidance and suppression were related to higher levels of psychopathology in children (Compas et al., 2017). Another meta-analysis built upon this work by assessing longitudinal studies and found evidence that the use of emotion regulation (e.g., using maladaptive strategies) could be a risk factor in the development of psychopathology (Cavicchioli et al., 2022). Additionally, one study found that the use of maladaptive strategies such as avoidance, and decreased use of adaptive strategies such as cognitive reframing was related to symptoms of social anxiety disorder (Werner et al., 2011). Vanderlind et al. (2022) found that emotion regulation was related to depression in that symptoms of depression contributed to the use of regulation strategies that did not promote positive emotion. However, the relationship between emotion regulation and psychopathology is not entirely clear. One study found that participants who reported greater use of worry and rumination, as well as the use of all types of emotion regulation strategies, had more symptoms of psychopathology than participants who used adaptive strategies but did not use all emotion regulation strategies (Dixon-Gordon et al, 2014). Although there is extensive research on how emotion regulation relates to psychopathology, more research is needed for investigating different types of moderators that may affect this relationship.

Emotion regulation is thought to relate to NED. However, the relationship is unclear. An earlier study found an association between higher NED and better emotion regulation in addressing negative emotions (Barrett et al., 2001). However, other studies have found different results. For example, in the face of stress, Brown et al. (2021) found that higher NED was related to less disengagement

strategy use (e.g., substance use) but not more constructive strategy use (e.g., instrumental). Another study found that less NED was not related to strategy selection, but was related to a decrease in the effectiveness of both adaptive and maladaptive strategy use for reducing negative emotions (Kalokerinos et al., 2019).

NED is also thought to relate to psychopathology. Specifically, NED has been found to be related to symptoms of depression (Demiralp et al., 2012; Erbas et al., 2014; Starr et al., 2017). There also seems to be some evidence to suggest that individuals experiencing social anxiety disorder (SAD) differentiate between negative emotions less frequently than those who do not have these symptoms (Kashdan & Farmer, 2014). Another study found that individuals with higher levels of trait anxiety struggled more with NED than did individuals with lower levels of trait anxiety (Matt et al., 2016). However, research on how NED relates to different anxiety disorders is limited.

In the literature, the relationships between all three of these topics (i.e., emotion regulation, NED, and psychopathology) have been explored. One study of adolescents found that higher emotion differentiation could serve as a protective factor against symptoms of depression and anxiety by means of more adaptive emotion regulation (Nook et al., 2021). Another study found that individuals with lower NED who were also considered highly socially anxious used less cognitive reappraisal than individuals who had a lower amount of social anxiety (O'Toole et al., 2014). However, there are currently no publications on the relationship among all three of these topics in children younger than age 14 (Nook, 2021). This highlights a gap in the literature that needs to be addressed. As early adolescents can experience symptoms of psychopathology (Pfeifer & Allen, 2021; Graber, 2013), it is important to investigate the developmental roots of these symptoms. If NED is found to be a moderator in the relationship between emotion regulation and psychopathology, this insight can provide a foundation for a prevention approach for children at risk of psychopathology such as techniques that aim to improve children's awareness of their emotions. These strategies could then contribute to more adaptive emotion regulation, and thus, could contribute to less intensity and frequency for symptoms of psychopathology.

Psychophysiological processes related to emotion differentiation, emotion regulation, and psychopathology are important to investigate, as they can provide another perspective to explore NED. Respiratory sinus arrhythmia (RSA) refers to how the heart rate relates to breathing, and it is thought to be a biological marker of the parasympathetic nervous system as well as an index for measuring an individual's emotion regulatory capacity (Bernston et al., 1997; Porges, 2007). RSA has been found to relate to emotion regulation; higher RSA is possibly associated with better emotion regulatory capacity, though results are somewhat mixed (Blandon et al., 2008; Fox et al., 2018; Gentzler et al., 2009). However, the relationship between RSA and NED has not been sufficiently explored in the literature. Some studies have found a possible positive correlation between emotion granularity, a concept that is synonymous with emotion differentiation, and RSA (Hoemann et al., 2020; Hoemann et al., 2021). However, both of these samples consisted of adult participants. The relationship between respiratory sinus arrhythmia and emotion differentiation in children has not been explored.

Resting RSA has been found to be negatively related to psychopathology (Zhang et al., 2017; Yaptangco 2015), though results are somewhat mixed (Beauchaine 2019). Another study found similar results, where participants who did not regularly engage in cognitive reframing had a negative correlation between RSA and worry and rumination (Plate et al., 2020). This presents another route of research that should be explored further.

Does Children's Negative Emotion Differentiation Relate to Emotion Regulation and Psychopathology?

Current Study

The current study seeks to replicate the findings from previous studies with a child sample. The aim of this project is to investigate whether NED in children is related to (1) the selection of adaptive (e.g., instrumental and support-seeking) emotion regulation strategies, (2) maladaptive (e.g., avoidance and suppression) emotion regulation strategies, (3) the effectiveness of the selected strategies in reducing unwanted emotions, (4) respiratory sinus arrhythmia, and (5) symptoms of depression and anxiety. NED will be investigated as a moderator among the selection of adaptive and maladaptive regulation strategies and internalizing psychopathology (i.e., symptoms of depression and anxiety). NED will also be investigated as a moderator in the relationship between the effectiveness of regulation strategy use and psychopathology.

METHODS

Participants

The sample for this study consisted of 106 pre-adolescent participants (59 males, *M* age = 9.3). Their ages ranged from 7 to 11 years old. Half of the sample had a family income of $40,000 or less. Participants' racial backgrounds were reported as Caucasian (17.9%), African-American (13.2%), Hispanic (31.1%), Asian-American (0.9%), other (0.9%), and mixed (35.8%). Participating children and one parent visited the Emotion Regulation Lab located on the main campus of University of California, Riverside. Participants only came to the lab for a single session. Parents provided consent and children provided assent before study procedures began. Families received a small honorarium for completing the study.

Measures

Negative Emotion Differentiation (NED)

NED was computed from emotion self-reports obtained from participants. The reports asked participants how strongly they felt a specific emotion. The emotions included happiness, anger, sadness, and fear. The intensities of these emotions ranged from one to four. These self-reports were administered to children five times throughout their visit. For the purposes of this study, only negative emotions were included in analyses which were anger, sadness, and fear. To obtain a score for NED, an average variance (Mikhail et al., 2020) was computed by taking the variance of these three negative emotions at each time point. These values were then averaged across time points to produce composite scores.

Emotion regulation

One of the measures used for assessing emotion regulation is an autobiographical emotion interview, which asked the participants to recall specific events that elicited fear, anger, and sadness (Davis et al., 2010). In these interviews, children were asked what emotion regulation strategies they used to cope with these negative emotions. For the current study, five emotion regulation strategies were assessed. Two of the strategies that were included were categorized as adaptive based on prior research done with US populations. These included cognitive reframing (e.g., a child thinking about the situation in a more constructive way) and social support seeking (e.g., a child asking a parent for help). The other three strategies that were included are considered to be maladaptive in prior literature with samples from the United States. These included avoidance and withdrawal (e.g., a child avoiding or leaving a situation), thought suppression (e.g., a child trying to inhibit how much they are experiencing specific thoughts), and expressive suppression (e.g., a child not physically expressing their emotions). For the purposes of this project, all responses to the three maladaptive strategies were composited into one maladaptive score.

To measure emotion regulatory capacity, the present student used biopsychological data regarding respiratory sinus arrhythmia (RSA). For this sample, RSA was measured noninvasively using an electrocardiogram (ECG) while children quietly performed neutral tasks such as reading a book or coloring (Quiñones-Camacho & Davis, 2017). This

took place for five minutes during the start of the visit. Children's RSA was quantified via 30-second epochs, and approximately ten epochs were recorded for each child during the initial baseline task. After collecting this data, resting RSA was computed by averaging epochs.

Child Psychopathology

The present study used two measures of psychopathology that were completed by the participants' parents regarding their children's symptoms. To measure symptoms of anxiety, the parent version of the Screen for Child Anxiety Related Emotional Disorders (SCARED) was administered (Birmaher et al., 1997). This measure consists of 41 items that ask parents to consider symptoms their child has experienced in the past three months. There are several scales included in this measure; panic disorder and somatic symptoms (e.g., "When my child feels frightened, it is hard for him/her to breathe"), generalized anxiety disorder (e.g., "My child worries about other people liking him/her"), separation anxiety disorder (e.g., "My child gets scared if he/she sleeps away from home"), social anxiety disorder (e.g., "My child doesn't like to be with people he/she doesn't know well), and school avoidance (e.g., "My child worries about going to school"). For each item, parents are asked to respond using a three-point scale: 0 (*not true or hardly true*), 1 (*somewhat true or sometimes true*), and 2 (*very true or often true*).

The second measure that was used in this project is the MacArthur Health Behavior Questionnaire (HBQ) which includes questions about physical health, mental health, and social and school functioning (Essex et al., 2002). The questionnaire included 170 items that asked parents to respond using a three-point scale: 0 (*never or not true*), 1 (*sometimes true*), and 2 (*often or very true*). This measure of internalizing behaviors is further divided into three subscales: depression (e.g. "Feels worthless or inferior), feeling overanxious (e.g., "Worries about things in the future"), and separation anxiety (e.g., "Worries about being separated from loved ones"). For each subscale, an average was computed by taking the mean of the responses.

RESULTS

Relationship Between NED, Psychopathology, and Adaptive Regulation Strategy Use

Results revealed that there was no relationship between NED and psychopathology. There was also no significant relationship between NED and the SCARED questionnaire, which includes generalized anxiety disorder, separation anxiety, social anxiety, school avoidance, panic, and a total anxiety score, $r(99) = \leq 0.11$, $p \geq 0.25$. The internalizing subscale from the HBQ also did not find significant results, $r(98) = 0.03$, $p = 0.75$. Results indicated that for children who did not use support seeking, there was a positive relationship between NED and separation anxiety, $r(45) = 0.29$, $p = 0.04$. Unexpectedly, an independent samples t-test revealed children who did not use cognitive reframing ($M = 0.23$, $SD = 0.36$) had higher NED than children who did ($M = 0.10$, $SD = 0.11$), $t(78) = 2.46$, $p = 0.01$. These results are represented in **Figure 1**.

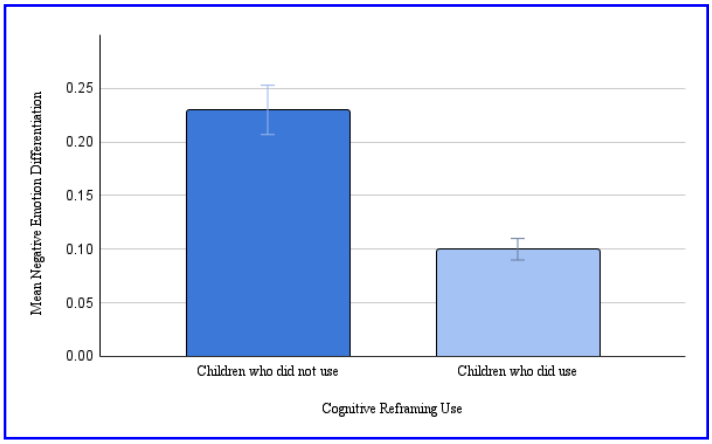

Figure 1 Participants' Mean Negative Emotion Differentiation and Cognitive Reframing Use

Differences in negative emotion differentiation between children who use cognitive reframing and children who do not ($N = 79$). The x-axis represents if children used cognitive reframing. The y-axis represents the childrens' mean negative

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emotion differentiation.

Respiratory Sinus Arrhythmia

An additional investigation was conducted regarding how respiratory sinus arrhythmia (RSA) was related to NED, ER, and psychopathology. Results in **Table 1** indicated that a higher resting RSA was related to fewer symptoms for some types of anxiety, but it depended on regulation strategy use.

CONCLUSION

The present study investigated if children's negative emotion differentiation (NED) was related to emotion regulation and internalizing psychopathology (i.e., depression and anxiety). Results from the study were mixed in terms of support for the hypotheses. First, NED and psychopathology were not correlated. Additionally, the analysis calculated an intraclass coefficient (ICC) to assess NED. As the data in the present study drew upon previously collected data for another research aim, the present study could only include a self-report scale of emotion with four items. Additionally, the analyses were conducted using the average variance of scores. Data for the second hypothesis found that NED was higher for children who did not use cognitive reframing. This is consistent with Kalokerinos et al., 2019, which found that emotion differentiation was related to decreased use of reappraisal. This result is possibly due to a risk of rumination.

Psychopathology Symptoms	General	Cognitive Reframing	
		Did Use	Did Not Use
Internalizing	$r(98) = -.18, p = .05$*	$r(36) = -.40, p = .01$**	$r(60) = -.09, p = .45$
Generalized Anxiety Disorder	$r(97) = -.22, p = .02$**	$r(37) = -.32, p = .04$**	$r(59) = -.13, p = .32$
Panic Disorder	$r(97) = -.34, p < .001$***	$r(37) = -.41, p = .008$**	$r(58) = -.26, p = .03$**
School Avoidance	$r(98) = -.19, p = .05$*	$r(38) = -.25, p = .11$	$r(59) = -.12, p = .34$
Social Anxiety	$r(98) = -.16, p = .09$	$r(38) = -.07, p = .63$	$r(58) = -.25, p = .04$**
Separation Anxiety	$r(98) = -.16, p = .09$	$r(37) = -.37, p = .01$***	$r(59) = .01, p = .88$
Total SCARED	$r(97) = -.27, p = .006$**	$r(38) = -.34, p = .03$**	$r(59) = -.20, p = .12$

Psychopathology Symptoms	Support Seeking		Maladaptive Strategies	
	Did Use	Did Not Use	Did Use	Did Not Use
Internalizing	$r(48) = -.26, p = .06$*	$r(48) = -.22, p = .12$	$r(26) = -.27, p = .16$	$r(70) = -.23, p = .04$**
Generalized Anxiety Disorder	$r(49) = -.29, p = .03$**	$r(47) = -.11, p = .45$	$r(25) = -.12, p = .55$	$r(71) = -.27, p = .01$**
Panic Disorder	$r(49) = -.52, p < .001$***	$r(47) = -.13, p = .35$	$r(25) = -.29, p = .14$	$r(71) = -.38, p < .001$***
School Avoidance	$r(49) = -.28, p = .04$**	$r(47) = -.14, p = .31$	$r(25) = -.06, p = .75$	$r(71) = -.26, p = .02$**
Social Anxiety	$r(50) = -.22, p = .11$	$r(47) = -.09, p = .54$	$r(25) = -.26, p = .18$	$r(72) = -.14, p = .20$
Separation Anxiety	$r(50) = -.19, p = .16$	$r(47) = -.12, p = .38$	$r(25) = -.14, p = .49$	$r(72) = -.18, p = .11$
Total SCARED	$r(49) = -.34, p = .01$**	$r(47) = -.16, p = .26$	$r(25) = -.26, p = .19$	$r(71) = -.29, p = .01$**

Table 1. Correlations Between RSA and Psychopathology Based on Regulation Strategy

Note: RSA = respiratory sinus arrhythmia
* Marginally significant
** p ≤ .05
*** p ≤ .001

For example, children may not find it effective to think about circumstances that had contributed to negative emotions, as this may lead to a cycle of repetitive negative thoughts about the event. If the child is experiencing different negative emotions, thinking about them can be even more overwhelming in the moment rather than being helpful. Data for the third hypothesis found NED was positively associated with separation anxiety for no support seeking. Experiencing more negative emotions may result in further discouragement of support seeking behaviors. Lastly, data for the additional hypothesis found that higher resting RSA is possibly a protective factor against some facets of psychopathology but depended on regulation strategy use. This implies that there are individual biological differences that may promote the effectiveness of some regulation strategy use. This approach could be used to inform children about which emotion regulation strategies may be more effective based on their psychopathology and resting RSA.

Limitations of the study include methodological boundaries. Prior studies used a daily diary method as a measure for NED, and the data was evaluated using an intraclass correlation (ICC). This differed from the measure and statistical procedure used for this study. Thus NED may not have been fully captured because the sample data had not been collected with the intention of measuring NED. Another limitation includes the assessments not being able to capture complex emotional states (Brown et al., 2021) such as feeling simultaneously anxious and excited. Another limitation includes that the measures were not ableto capture cultural and ethnic differences. Lastly, these measures were not able to capture within-emotion differentiation (e.g., anger, frustration, and irritability).

Future directions can address these limitations as well as explore how NED, emotion regulation, and psychopathology may otherwise be related. This can inform clinical interventions that aim to increase NED to improve the frequency and effectiveness of adaptive emotion regulation strategies. Doing so can help mitigate risks of developing (or experiencing more intensely) conditions associated with psychopathology.

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Change in protease gene expression in an insect pest to plants in the potato/tomato family (Solanaceae)

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ABSTRACT

Lineage-specific gene expansions may allow insects to adapt and diverge functional traits. The Hansen lab's chromosomal assembly of the sap-sucking potato psyllid (*Bactericera cockerelli*) previously identified three rapidly evolving genes associated with protease expression. The current study further analyzed whether or not plant diet and insect life stage might impact protease expression. We fed insects different plant diets of either potato or tomato at two insect life stages (2nd instar and young adult) to test for differences in insect protease gene expression. First, RNA extractions were conducted, followed by quantitative PCR (qPCR). The study used the Δ-Δ Ct method to analyze the relative comparison of gene expression between two genes and the ANOVA test to determine if there is a relationship between the changes in gene expression. Preliminary data revealed a significant difference between the host plant diets potato and tomato for 2nd instar nymphs for two of the three candidate genes. This project begins laying the foundation for identifying the function of species-specific protease genes that are evolving rapidly in the insect, and ultimately these genes may be involved in host plant adaptation.

KEYWORDS: *Bactericera cockerelli*, lineage-specific gene expansion, carboxypeptidase, quantitative PCR, delta-delta Ct method, ANOVA test

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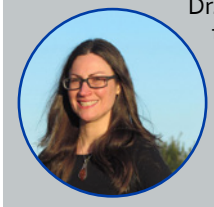

Dr. Hansen is an Associate Professor in the Department of Entomology at the University of California, Riverside. She is interested in ecological and evolutionary genomics of insect-microbe interactions to provide novel insights into the complexity and dynamics of insect-microbe symbioses. Her research investigates the molecular mechanisms that underpin insect-microbe co-evolution using comparative and functional genomics in an evolutionary framework.

Dana Morshed

Dana Morshed is a fourth year Biology Major. She has been researching under Dr. Hansen since her sophomore year. She has received funding from the UC Riverside Undergraduate Education Mini Grant program and presented her research at the Southern California Conferences for Undergraduate Research. She is a member of the University Honors program and serves as an Honors Ambassador. In addition to being involved in various clubs on campus, she is currently a volunteer at a free clinic and also works as a medical scribe. She plans to pursue a career in medicine.

Change in protease gene expression in an insect pest to plants in the potato/tomato family (Solanaceae)

INTRODUCTION

Lineage-specific gene expansions are the new appearance of genes in a lineage that is not in a sister lineage (Jordan et al., 2001). These lineage-specific genes are involved in pathogen interactions and stress resistance. (Lespinet et al., 2002). Lineage-specific gene expansions allow eukaryotes to adapt and are regarded as a critical reason for organizational and regulatory diversity (Lespinet et al., 2002).

A recent study from my laboratory found several gene expansion events in an insect called *Bactericera cockerelli* (Kwak et al., 2022). This insect is known to house and vector microbes (Hansen et al. 2008) and belongs to the insect group called psyllids, which are small, plant sap-sucking insects that fly and are often recognized as pests on agricultural plants because of their ability to vector bacterial plant diseases (Trumble et al., 2011). This psyllid species feeds on the *Solanaceae* or nightshade plant family, including potatoes and tomatoes (Pletsch, 1947). This study focuses on these gene expansions to ultimately better understand host-plant relations.

The first chromosomal assembly of the psyllid *B. cockerelli* from my laboratory found that *B. cockerelli* has significantly more gene expansion events than other hemipteran species examined. This study noted that 75% of these gene expansions are transposable elements found throughout *B. cockerelli's* chromosomes which may contribute to the large size of the genome relative to other psyllids sequenced (Kwak et al., 2022). For genes that could be annotated, several gene expansion events included genes associated with transcription factors, proteases, and odorant receptors (Kwak et al., 2022). For example, gene expansions of one protease family were predicted to be related to carboxypeptidase D (Kwak et al., 2022). Proteases are enzymes that break peptide bonds (Bond, 2019). Carboxypeptidases are proteases that remove the C-terminal amino acid from proteins (NIH, 1999). These carboxypeptidases play a vital role in various physiological functions in organisms (Sapio & Fricker, 2014). I hypothesize that the expansion of protease gene families in *B. cockerelli* is important because of its impact on species-specific responses to host plant defenses. For example, both tomato and potato plants, which are the psyllid's host plants, produce carboxypeptidase inhibitor proteins in response to insect feeding (Díez-Díaz et al., 2004; Graham & Ryan, 1981). Since these inhibitors can cause antifeedant effects for insects (Zhu-Salzman & Zeng, 2015), *B. cockerelli* may have an evolutionary arms race of rapidly evolving carboxypeptidase families in response to solanaceous host plant defenses (Kwak et al., 2022).

This study analyzes if there are insect life stage and host plant feeding differences in insect gene expression for three rapidly evolving genes in *B. cockerelli*. It is hypothesized that these proteases play a critical role in host-plant interactions and are significant in responding to host-plant feeding. Based on their function, certain genes are expressed in varying abundances throughout different insect life stages, so this study analyzed both nymphs and adults. Since the function of the candidate protease genes examined here is unknown, it is important to determine if there is a difference in gene expression. Furthermore, although *B. cockerelli* feeds on potatoes and tomatoes, it may exhibit different responses to each host. A significant difference in expression between life stages for all candidate protease genes on both host plants was predicted. Gene expression was quantified using quantitative polymerase chain reaction (qPCR) to test this hypothesis. Identifying and understanding these unknown genes and their functions can advance our knowledge of the psyllids' relationship with plants. Understanding this relationship is vital because psyllids impact agriculture since they are considered pests.

MATERIAL AND METHODS

This approach involved first growing colonies of psyllids from the same genetic line on plant diets of potato and tomato plants. The psyllids were obtained from the University of California (UC), Riverside, from the same genetic line

as the published genome (Kwak et al., 2022). The colony was confirmed to be free of the plant pathogen *Candidatus Liberibacter psyllaurous* (Hansen, 2008) via Real-time PCR as described in Kwak et al. (2022).

The two established lines were maintained on 12-week-old *Solanum tuberosum* (Russet potato) and *Solanum lycopersicum* (Moneymaker tomato) plants at 25°C under a 16L:8D light/dark cycle in an incubator. After three generations of rearing on either tomato or potato plants, the colony stabilized and was ready for experiments. I used mesh cages, tomato and potato seeds, soil, pots, and planting materials to grow and maintain the colonies, as detailed in Kwak et al. (2022).

Three biological samples (replicates) of 2nd instar nymphs and young adults were randomly selected per host plant treatment, totaling 12 samples. For 2nd instar nymphs, 20 insects were pooled, and for young adults, four were pooled per biological replicate. The life stages of the psyllids were confirmed under the microscope before pooling. Specifically, the psyllids were tracked daily by recording the number of psyllids and their life stage. After hatching from the egg, the nymph was in the 1st instar stage for around three days and then stayed in the second for approximately 2.5 days (Knowlton and Janes, 1931). After about 13 days, the psyllids became young adults. The stages were monitored by looking at the leaves of the plants with nymphs under a microscope.

Psyllid samples were extracted for total RNA. The extractions were performed with the Zymo Quick-RNA Miniprep kit (Zymo Research, Irvine, CA, USA) and DNase 1 treated to remove DNA contamination from samples. RNA was purified using the RNA Clean & Concentrator-5 kit (Zymo Research, Irvine, CA, USA). The concentration and quality of the RNA were checked using the SpectraMax QuickDrop Micro-Volume Spectrophotometer (Molecular Devices, San Jose, CA, USA).

Reverse transcription was then used to create cDNA from the RNA. This was done using the iScript™ Reverse Transcription Supermix with the Bio-Rad C1000 Thermal Cycler with Dual 48 Well Block machine (Bio-Rad Laboratories, Hercules, CA). The cDNA was then used for quantitative polymerase chain reaction (qPCR) to examine gene expression. Three technical replicates were run for qPCR with similar reagents and conditions for each biological replicate, as described in Kwak et al. (2022). Three plates were run to analyze each gene of interest alongside the housekeeping gene on each plate.

The Δ-Δ C_t or comparative C_t method was used to analyze the qPCR data results. The fold change of gene expression was calculated relative to the calibrator (Bookout, 2006). In this case, the calibrator was the adult treatment group to determine if expression differed in the 2nd instars for each host plant. For host plant comparisons, tomato adults were used as the calibrator to determine if there was a difference in expression among all treatment types. IBM SPSS Statistics Program (Version 28) was used to test for significance in the difference in gene expression. A two-way ANOVA test was used with *post hoc* analysis.

RESULTS

Candidate protease gene ANN00645:

When examining the fold change of gene expression relative to tomato adults among all treatment groups (**Figure 1**), using a two-way ANOVA test, there is no significant difference ($P>0.05$) in the expression of the gene (ANN00645) between host-plant treatments or life stages.

Change in protease gene expression in an insect pest to plants in the potato/tomato family (Solanaceae)

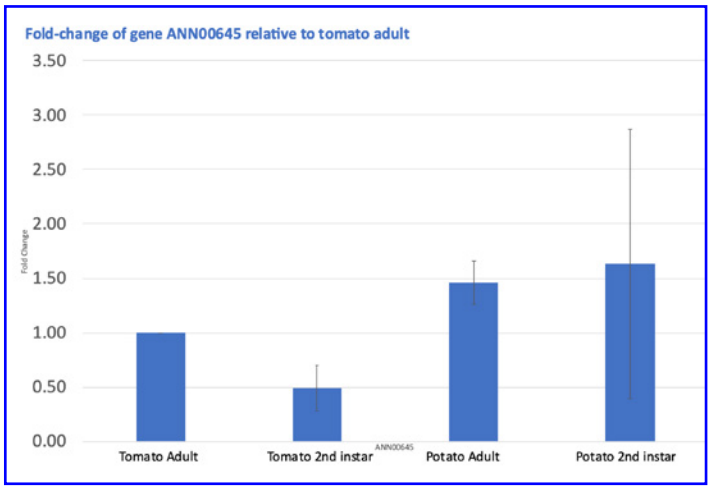

Figure 1. Fold change of Gene ANNoo645 relative to the tomato adults among all treatments

Candidate protease gene ANN17948:

When examining the fold change of gene expression relative to tomato adults among all treatment groups (**Figure 2**), using a two-way ANOVA test, there is a significant difference in the expression of the gene (ANN17948) between host-plant treatments (P=0.028), but not life stages (P>0.05). Compared to potatoes at the same life stage, a lower candidate protease gene expression level occurred when psyllids fed on tomato at 2nd instar.

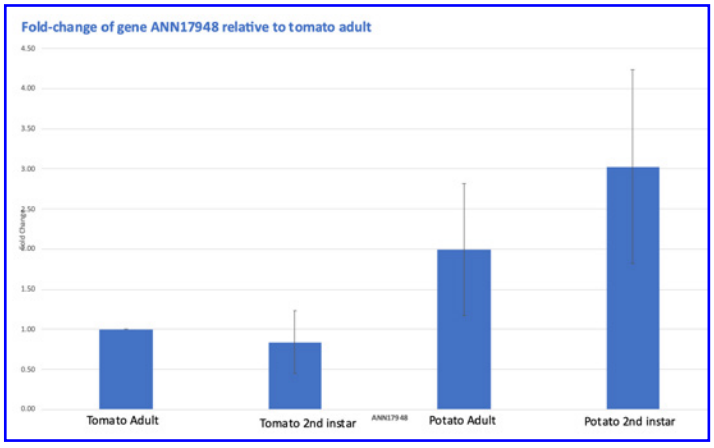

Figure 2. Fold change of Gene ANN17948 relative to tomato adults among all treatments

Candidate protease gene ANN17915:

When examining the fold change of gene expression relative to tomato adults among all treatment groups (**Figure 3**), using a two-way ANOVA test, there is a significant difference in the expression of the gene (ANN17915) between host-plant treatments (P=0.018), but not life stage (P>0.05). In sum, a lower gene expression level occurred when the psyllid fed on tomato at the 2nd instar compared to all other treatments.

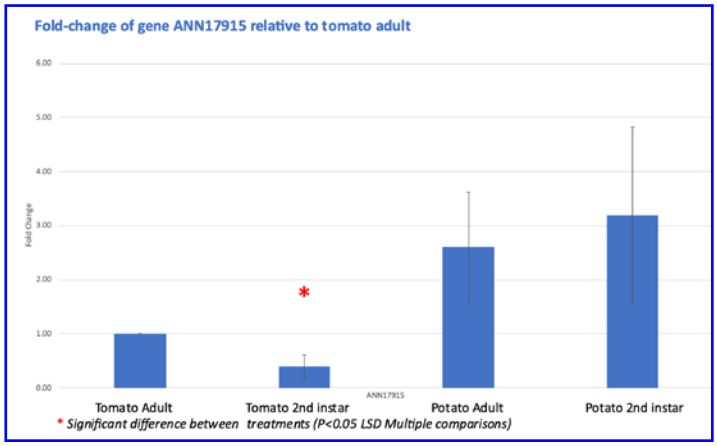

Figure 3. Fold change of Gene ANN17915 relative to the tomato adults among all treatments

DISCUSSION

When comparing the fold change of psyllid gene expression for three rapidly evolving protease gene candidates, my preliminary data suggests a significant difference between host plant treatment for candidate protease genes ANN17948 and ANN17915. The difference in gene expression between the two host plants shows that these genes may have a more specific host plant response to feeding, especially at 2nd instar.

This difference in gene expression for genes ANN17948 and ANN17915 may be attributed to the evolutionary history of tomato and potato plants. Although research is ongoing, differences in host plant history can be credited

to past hybridization events and varying speciation rates (Rodriguez et al., 2009). Furthermore, studies on tomato defense mechanisms show that regulators of gene expression are largely responsible for defensive responses to pathogens (Campos et al., 2022). Future studies can further narrow down the function of these psyllid protease genes through methods such as RNA sequencing and studies involving RNAi and CRISPR. Also, future studies should use larger sample sizes to reduce variation.

In conclusion, two candidate genes of *B. cockerelli* (Kwak et al., 2022) displayed differential gene expression (**Figures 1-3**). These gene candidates may be involved in host plant adaptation which has implications across multiple disciplines, including evolution, entomology, and agriculture. Studying these newly discovered genes adds to our understanding of the putative function of species-specific protease genes that are evolving rapidly in *B. cockerelli* (Kwak et al., 2022). In future studies, it is essential to deduce what these genes do to further elucidate insect-plant evolution and the psyllids' role as an agricultural pest.

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I would like to thank my wonderful faculty mentor, Dr. Allison K. Hansen, for giving me the opportunity to learn and grow throughout my undergraduate career. I would also like to thank my graduate student mentor, Younghwan Kwak, for her guidance and patience. I want to thank the Mauck Lab for providing me with psyllids to begin my project. Finally, thank you to the UC Riverside Undergraduate Education Mini-Grant committee for funding this research.

Change in protease gene expression in an insect pest to plants in the potato/tomato family (Solanaceae)

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Effects of Acute Stress on Parental Behavior in Reproductively Naïve Male California Mice

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ABSTRACT

In many biparental mammalian species, such as California mice (Peromyscus californicus), new fathers exhibit affiliative behavior toward unfamiliar infants, whereas reproductively naïve males show highly variable behavioral responses to infants. The sources of this variation are unknown. We investigated the effects of acute stress on pup-directed behavior in reproductively naïve male California mice. Each mouse underwent three 10-minute tests with an unfamiliar pup at 48-hour intervals. Males in the stressed group (N=22) were stressed using subcutaneous oil injections, a common experimental stressor used in rodents, immediately before each of the first two tests. The controls (N=22) were left undisturbed to avoid any experimentally induced stress. Compared to controls, stressed mice spent significantly less time performing paternal behavior in tests 1 and 2, while only marginal differences were seen in test 3. In tests 2 and 3, significantly fewer stressed mice interacted with the pup than controls. These findings suggest that acute stress experienced by reproductively naïve males might contribute to both short-term and long-term differences in pup-directed behavior.

Key Words: Stress; reproductively naïve; California mouse; biparental; paternal behavior; subcutaneous injections; behavioral tests; experimental pups.

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Dr. Saltzman is a Professor in the Department of Evolution, Ecology, and Organismal Biology. She has a B.Aa in Animal Physiology from UC San Diego and a Ph.D. in Animal Behavior from UC Davis, and did her postdoctoral work at the University of Wisconsin, Madison. She returned to Southern California and joined the faculty of UCR in 2001. Dr. Saltzman's research focuses on neural and physiological consequences of parenthood and neuroendocrine influences on parental behavior in biparental rodents.

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Parihan Asif is a fifth-year Biochemistry and Cell, Molecular, and Developmental Biology major. In Dr. Saltzman's behavioral neuroendocrinology lab, Parihan focused on how stress and empathy are displayed in biparental species. Parihan has presented her research at UC Riverside, Stanford University, and Loyola Marymount University. Parihan plans to pursue a career in Emergency Medicine.

Effects of Acute Stress on Parental Behavior in Reproductively Naïve Male

INTRODUCTION

Parental care encompasses behaviors that positively contribute to the evolutionary fitness of the offspring (Klug and Bonsall, 2014). Mothers in all mammalian species provide parental care. In contrast, only about 6% of mammalian species exhibit biparental care, in which both fathers and mothers provide care for their offspring (Kleiman and Malcolm, 1981). In biparental mammals, fathers can provide comparable forms and amounts of care as mothers, excluding lactation (Kleiman and Malcolm, 1981), and usually care for unrelated infants during experimental exposure (Gubernick et al., 1987). Paternal care can be important for offspring survival and development in these species (Bales and Saltzman, 2016).

In biparental mammals, the onset of paternal behavior in new fathers is associated with changes in hormones, neuropeptides, and their receptors, which can be triggered by interactions with the pregnant or parturient mate and/or the offspring (Horrell et al., 2021). Reproductively naïve males–i.e., males that have never been housed with an unrelated female and therefore have not copulated or been exposed to pups–however, lack the sexual and parental experience needed to trigger these neuroendocrine changes and the onset of paternal care. Compared to fathers, adult reproductively naïve males exhibit more varied responses when exposed to an unfamiliar pup, performing nurturant, avoidant, or aggressive behavior (Elwood and Stolzenberg, 2020). The sources of this variation in initial responses to pups are not well understood, although reproductively naïve males that behave paternally toward pups may be less anxious than those that do not (Chauke et al., 2012; De Jong et al., 2012). Moreover, repeated exposure to pups can facilitate the onset of paternal care in reproductively naïve males (Horrell et al., 2017; Cai et al., 2020).

Numerous studies in humans, as well as several studies in other biparental mammals, have found that stress can negatively affect parental care and, in humans, can increase the likelihood of child abuse or neglect (Wolfner and Gelles, 1993; Lee et al., 2008). In contrast, virtually nothing is known about the possible effects of stress on responses to infants in reproductively naïve males. Our study aimed to characterize the impacts of stress on the onset of paternal behaviors specifically for reproductively naïve males in a biparental mammal, the California mouse (*Peromyscus californicus*). In this rodent, both parents provide intensive care for their offspring (Dudley, 1974; Gubernick and Alberts, 1987). Fathers spend as much time retrieving, grooming, and huddling their offspring as mothers, and care by fathers is important for the normal behavioral, neuroendocrine, and social development of pups (Bales and Saltzman, 2016).

In the present study, we investigated the long- and short-term effects of stress on the onset of paternal behavior in reproductively naive males. Previous work in our lab has found that in California mouse fathers, acute stress transiently reduces the amount of time spent interacting with the pups and mate (Harris et al., 2013). However, the effects of stress on responses to pups in reproductively naïve males have not been examined. We tested the hypothesis that stressors will inhibit the short-term expression of paternal behavior in reproductively inexperienced males. In addition, we tested the hypothesis that concurrent exposure to a stressor and pups, which constitute an unfamiliar stimulus, will cause reproductively naïve males to form an association between pups and stress, leading to longer-term effects on males' behavioral responses to pups.

METHODS

Animals

California mice were descended from mice purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, USA) and were bred and housed at the University of California, Riverside (UCR). Polycarbonate

cages (44 x 24 x 20 cm) were used to house the mice. The animals were kept under standard laboratory conditions (Nguyen et al., 2020), with aspen shavings for bedding and cotton for nesting, and had ad libitum access to food (Purina 5001 Rodent Chow) and water. Animals were removed from their parents' cages at weaning age (27-31 days), before the birth of their younger siblings, and housed in groups of 3-4 same-sex, age-matched mice. At least 2 weeks before being used in the study, groups were split into pairs of reproductively naïve males. All procedures were reviewed and approved by the UCR Institutional Animal Care and Use Committee and were consistent with the recommendations of the *Guide for Care and Use of Laboratory Animals*.

Design and Stimulus Exposure

We used 22 pairs of adult reproductively naïve males (143-168 days of age). Each mouse was tested with an unrelated pup three times at 48-hour intervals. Within each pair, one male was randomly assigned to the stressed group and the other to the control group (**Table 1**). The subjects in the stressed group received an injection of sesame oil immediately before the first and second tests but not the third test; whereas, the subjects in the control group did not undergo any experimental stressors prior to any of the tests. We chose to use oil injection as a stressor because it elicits a stress response in this species (as determined by an increase in plasma concentrations of the adrenocortical stress-responsive hormone corticosterone) beginning within 10 minutes and persisting for at least 40 minutes after injection (Harris et al., 2013) and therefore seemed likely to affect the mice's behavior during the entire test. We considered the entire procedure of injecting the stressed mice with oil (including handling, brief pain from the injection, and more prolonged discomfort from the oil) to constitute the stressor. Therefore, we did not perform any of these procedures on the control mice in order to avoid stressing them.

Group	Day 1: Test 1	Day 3: Test 2	Day 5: Test 3
Stress (N=22)	Acute stress + pup	Acute stress + pup	No stress + pup
Control (N=22)	No stress + pup	No stress + pup	No stress + pup

Table 1. Experimental design. Total sample size N=44.

At the beginning of each test, the subject was placed alone in a clean cage identical to the home cage. After a 20-minute acclimation period, mice in the stressed group were injected with sterile sesame oil (0.2 ml, s.c.); control mice were left undisturbed. An unrelated pup, 2-5 days of age, was then placed in the cage with the subject for 10 minutes. The pup was removed immediately if it was attacked. Cage mates were tested 5 minutes apart and were reunited in their home cage after each test. We conducted tests during the inactive (light) phase of the 24-hour light cycle (9:00-11:00 h).

Behavior Measurements

We video-recorded the mice throughout the 10-minute test period. Behaviors (**Table 2,** next page) were scored from the videos using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016). Inter-observer reliability was 91%, and observers were blind to animals' treatment conditions. Because the precise amount of time that pups were present varied slightly across tests, the time spent in each behavior was normalized by dividing the total time of the activity by the duration of the stimulus exposure and multiplying by 100 [Σ behavior (s) / stimulus presentation (s)*100].

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Behavior	Description	Measure
Sniff	Move nose against or in the direction of pup within 1 cm of pup	Duration, latency
Parental Behavior	Stand or lie over or against pup and/or lick pup	Duration, latency
Rest	Sit or lie for ≥2 s while not active and not in contact with the pup	Duration
Jump	Jump up and down	Duration
Proximity	Nose and/or abdomen within 5 cm of pup	Duration
Pick up/Carry	Hold pup in the mouth or hold and locomote	Frequency
Attack	Bite pup	Did or did not occur
Backflip	Flip in a vertical circular motion (a common stereotypical behavior in this species	Frequency
Autogroom	Use hands and/or mouth to pick through, wipe, or scratch its own body	Duration

Table 2. Behaviors scored

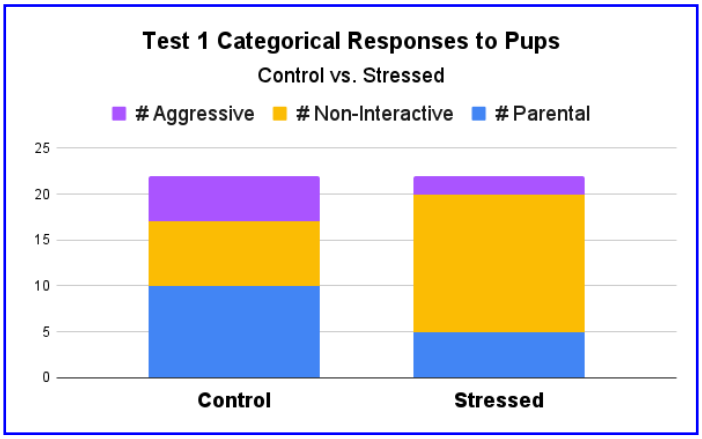
Statistical Analysis

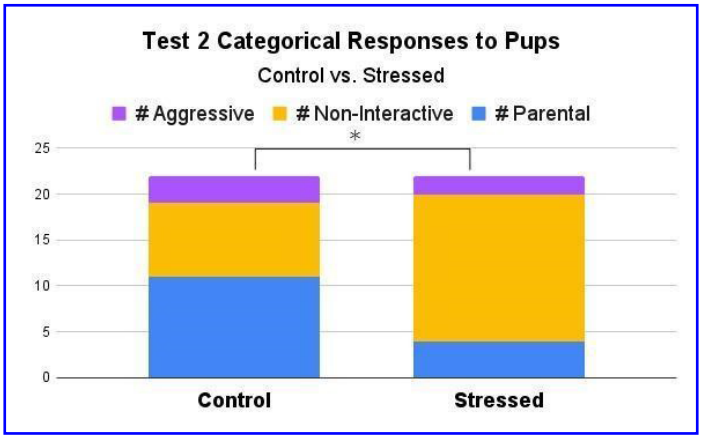
We analyzed data using non-parametric tests because measures did not meet the criteria for parametric analyses. Mann-Whitney U tests were used to compare behavior between stressed and control animals in each test, and Wilcoxon tests were used for within-subjects comparisons between tests 1 and 3 for each treatment group separately. For all analyses, we used a critical P value of 0.05 (2-tailed).

RESULTS

Comparisons of stressed and control groups

The number of reproductively naïve male California mice that exhibited parental (lick, groom, and/or huddle the pup), aggressive (attack the pup), or non-interactive behavior (neither parental nor aggressive) showed a marginally significant difference between the stressed and control mice in test 1 (P=0.053) and differed significantly between the two groups in tests 2 (P=0.047) and 3 (P=0.004; **Fig. 1**). In each test, stressed mice were more likely to be non-interactive and less likely to exhibit parental behavior than controls. Few





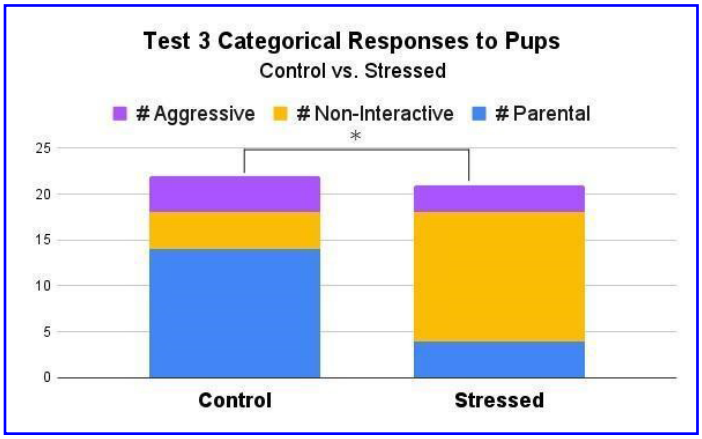
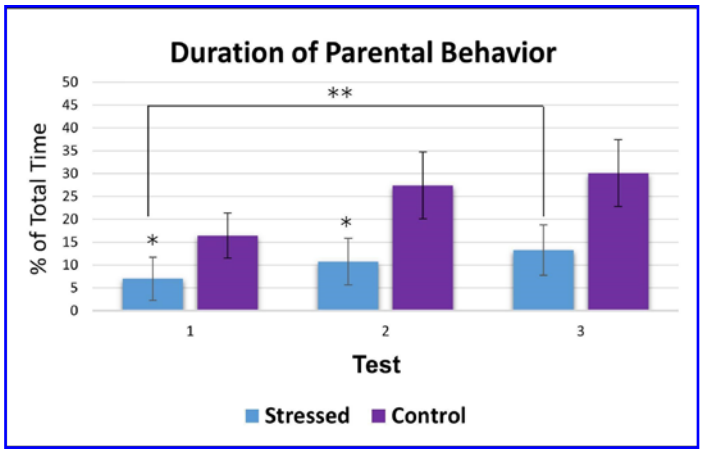


Figure 1. Numbers of stressed and control males that exhibited parental behavior, aggressive behavior, or neither in tests 1, 2, and 3. *P<0.05 for stressed vs. control mice. N=22/group except N=21 stressed males in test 3.

stressed or controlled males attacked the pup in any test.

Mice in the stressed condition spent significantly less time engaging in parental behavior, compared to controls, in test 1 (P=0.044) and test 2 (P=0.037; **Figure 2A**). The same pattern was seen in test 3, but the difference was not statistically significant (P=0.070). Duration of time spent in proximity to the pup did not differ between stressed and control mice in test 1 (P=0.142). However, stressed mice spent significantly less time in proximity to the pup in test 2 (P=0.015) and tended to spend less time in proximity in test 3 (P=0.060), compared to controls (**Figure 2B**). None of the remaining measures (see **Table 2**) differed between the stressed and control groups in any test.



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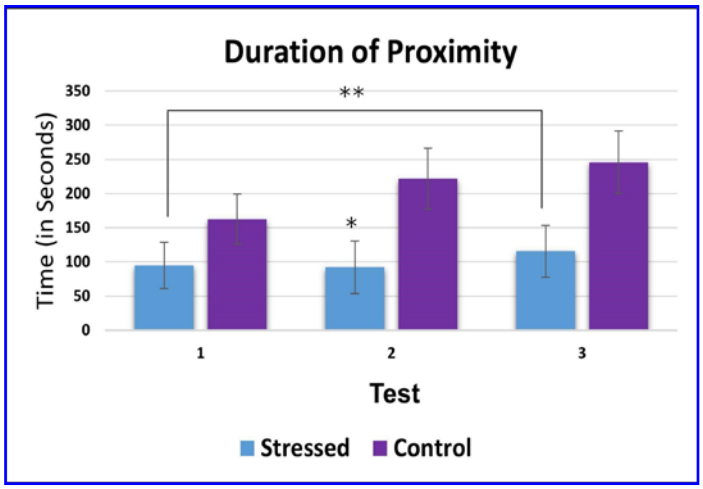

B

Figures 2A and 2B. Duration of time (mean ± SE) spent A) performing parental behavior, and B) in proximity to the pup by stressed and control mice in tests 1, 2, and 3. *P<0.05 for stressed vs. control mice. **P<0.05 for test 1 vs. test 3. N=22/group except N=21 stressed males in test 3.

Comparisons of tests 1 and 3

To determine how responses to pups changed with repeated exposure in mice that were and were not exposed to stress, we compared behaviors between test 1 (acute stress only) and test 3 (previous exposure to stress only) within each treatment group separately. We did not include test 2 in these analyses because, in this test, mice had both acute and previous exposure to stress.

Control mice spent significantly more time engaging in parental behavior ($P=0.0014$; **Figure 2A**) and more time in proximity to the pup ($P=0.028$; **Figure 2B**) in test 3 than in test 1, and were quicker to approach the pup ($P=0.028$; data not shown) and to initiate parental behavior ($P=0.036$; data not shown) in test 3 than in test 1. In contrast, stressed mice showed a significant change only in the duration of time spent grooming themselves, which decreased from test 1 to test 3 ($P=0.0088$; data not shown).

DISCUSSION

Fathers in biparental species, such as the California mouse, are strongly attracted to their offspring and actively engage in parental behavior, even toward unrelated infants (Elwood and Stolzenberg, 2020). Reproductively naive male California mice, on the other hand, show highly variable responses to experimentally presented pups, which can include nurturing, avoiding, or attacking the pups (Gubernick et al., 1994; De Jong et al., 2009; Horrell et al., 2017; Nguyen et al., 2020). The sources of this variation are not well understood. In this study, we evaluated the possibility that acute stress can affect reproductively naïve males' initial responses to unfamiliar pups and can have carryover effects on their subsequent behavior toward pups.

Effects of Acute Stress on Initial Responses to Pups

In their first test with a pup, mice that were injected with oil immediately prior to pup exposure spent significantly less time engaging in parental behavior (licking, grooming, huddling) and significantly more time grooming themselves, compared to non-stressed controls. They also tended to be less likely to perform parental behavior and less likely to interact with the pup at all, but these differences were only marginally significant. These results support our hypothesis that stress can have short-term inhibitory effects on parental behavior in reproductively naïve male California mice.

In test 2, as in test 1, oil-injected mice spent significantly less time performing parental behavior and more time grooming themselves, compared to controls. Additionally, in test 2, injected mice spent significantly less time in proximity to the pup and were slower to perform parental behavior than control mice. Oil-injected mice were also significantly less likely to perform parental behavior, or to interact with the pup at all, than non-stressed males in test 2. In this test, however, mice in the stress condition had been exposed to stress both immediately before the presentation of the pup and previously (i.e., in test 1); therefore, the results in test 2 cannot be attributed definitively to either acute or previous stress exposure.

Carryover Effects of Stress on Subsequent Responses to Pups

We found mixed support for our hypothesis that stress can have carryover effects on reproductively naïve males' responses to pups. In test 3, when neither group of males was stressed acutely, the two groups did not differ significantly in the durations or latencies of any behavioral measures. On the other hand, we found a highly significant difference between the stressed and control groups in the number of mice that engaged in parental, non-interactive, and aggressive behavior. As in test 2, stressed males were less likely than controls to engage in parental behavior and more likely to be non-interactive.

Because reproductively naïve male California mice exhibit increasing parental behavior upon repeated pup exposure (Horrell et al., 2017), we compared behavior in test 1 (acute

stress only) and test 3 (previous exposure to stress only) to determine if repeated exposure in mice leads to behavioral responses under stressed and non-stressed conditions. We found that in control mice, repeated exposure to pups resulted in significantly more time spent engaging in parental behavior, as in our previous study (Horrell et al., 2017). Additionally, control mice spent more time in proximity to the pup in their third test with a pup compared to their first test. However, the only change stressed mice showed was a significant decrease in auto-grooming from test 1 to test 3. This could be due to the fact that stressed mice did not receive an injection in test 3 and therefore might have spent less time auto-grooming the region of injection when compared to test 1. Thus, concurrent exposure to stress and a pup blocked the usual effects of repeated pup exposure on parental behavior in reproductively naïve males.

Interestingly, very few mice attacked the pup in any of the tests, and control mice were slightly more likely to attack than stressed mice. These findings suggest that stress does not have either acute or carryover effects on pup-directed aggression in reproductively inexperienced male California mice. Moreover, the lack of interest by the stressed reproductively naïve males towards the infants begs the question of whether the effect is pup-specific or reflects a general lack of interest in stimuli caused by the oil injections. To test this question, we are currently replicating the study using virgin females as stimuli instead of pups.

CONCLUSIONS

In conclusion, this study is the first, to our knowledge, to experimentally investigate the effects of stress on reproductively naïve males' responses to pups in a biparental mammal. We found that acute stress reduces paternal behavior and increases non-interactive behaviors in reproductively inexperienced adult males, in both the short and longer terms. Our findings provide new insights into the possible sources of variation in reproductively naïve males' responses to pups and, potentially, into the onset of parental care in new fathers.

Effects of Acute Stress on Parental Behavior in Reproductively Naive Male

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Childhood Emotional Abuse, Caregiver Attachments, & Self-Worth: Mechanisms in the Cycle of Violence

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ABSTRACT

The cycle of violence hypothesis holds that experiencing abuse in childhood increases the risk of perpetrating or experiencing violence, particularly in young adulthood. Research suggests that childhood maltreatment can increase children's risk for later violence, particularly in intimate relationships, by weakening their attachments to primary caregivers and lowering their sense of self-worth. Extant research has focused primarily on childhood physical abuse and exposure to intimate partner violence (IPV), leaving a gap in our understanding of if and how childhood emotional abuse (CEA) contributes to later violence. This study sought to fill that gap by evaluating if CEA predicted young adult IPV perpetration and victimization via compromised attachment to caregivers and self-worth. The current sample of 1,091 college students (Mage = 19.23, SD = 1.65; 68.4% female sex assigned at birth) was ethnically and racially diverse (i.e., 41.9% Asian, 26.5% Latine, 15.9% White, 5.9% Black, and 9.9% multiracial/other). Participants completed surveys in a research lab using a confidential online platform to assess their childhood maltreatment experiences, caregiver attachments, self-worth, and IPV perpetration and victimization. Multiple mediation analyses controlling for sex assigned at birth and childhood IPV exposure showed significant effects from CEA to IPV perpetration and victimization via decreased self-worth but not compromised attachment. Follow-up analyses by the sex assigned at birth showed an indirect pathway from CEA to IPV perpetration through compromised caregiver attachment for females. Consistent with prior data suggesting more substantial effects for females, CEA contributed to IPV victimization for females through decreased self-worth but not for males. These findings highlight the importance of considering sex-specific pathways from CEA to IPV. Our results also illuminate self-worth as a modifiable target of interventions to stem the cycle of violence and attachment security to prevent IPV perpetration among females.

KEYWORDS: attachment, emotional abuse, intimate partner violence, mediation, self-worth

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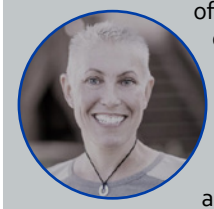

Dr. Yates is Professor of Psychology and the director of Adversity & Adaptation Laboratory. She holds a joint doctorate in clinical and developmental psychology from the Institute of Child Development, University of Minnesota, Twin Cities. Dr. Yates' research examines how children are affected by, and in many cases successfully negotiate, different kinds of adverse life experiences, such as poverty, community and family violence, other traumas, loss, and illness. Her work seeks to understand how and why negative life experience undermines the development of some children, while others are relatively less affected. Her work has also been supported by the National Science Foundation, the National Institute for Child and Human Development, and the Haynes Foundation. Dr. Yates is the founder and executive director of the UCR Guardian Scholars, a program that supports emancipated foster youth attending UCR.

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INTRODUCTION

Scholars have proposed different theories to explain the effects of childhood maltreatment (i.e., before age 18) on later physical and mental well-being. The cycle of violence theory holds that experiencing childhood maltreatment increases the risk of perpetrating or experiencing violence in adulthood (e.g., Ehrensaft et al., 2003). Consistent with this theory, studies show that all forms of childhood maltreatment can predict later intimate partner violence (IPV; e.g., Curtis et al., 2022). However, fewer studies have considered the effects of childhood emotional abuse (CEA; i.e., acts that belittle, threaten, isolate, corrupt, or neglect children and their needs for emotional security; see Hibbard et al., 2012) on IPV perpetration and victimization. In a study of 184 adolescents, Zurbriggen et al. (2010) found that CEA was the strongest predictor of sexual aggression perpetration compared to other forms of maltreatment. However, other studies suggest that these findings may be sex-specific. For example, in a US survey of over 12,000 middle and high school students, CEA predicted IPV perpetration and victimization for females but not males (Richards et al., 2017). CEA remains underreported and understudied in maltreatment literature, likely due to the lack of a uniform legal definition and poor consensus around what constitutes CEA (Taillieu, 2016; Trickett et al., 2009). Thus, the present study filled these gaps by evaluating the cycle of violence hypothesis from CEA to later IPV as explained by two specific and modifiable developmental processes: attachment to caregivers and self-worth.

Attachment theory (Bowlby, 1973) posits that inconsistent or unresponsive caregiving, commonly experienced by maltreated youth, hinders the development of secure attachments in ways that compromise the safety and security of subsequent close relationships (e.g., Davies & Cummings, 1994; Reckdenwald et al., 2014). A sense of security increases children's capacities to regulate their emotions and behavioral expressions of emotion (Davies & Cummings, 1994), which is necessary for safely and successfully navigating conflict in interpersonal relationships. Thus, we hypothesized that CEA would precipitate insecure attachments to caregivers that undermine later relational coping skills and perpetuate IPV.

Given that children's self-worth also develops in the context of the early caregiving environment (Harter, 2012), we further hypothesized that CEA would undermine children's self-worth in ways that engender later IPV. Indeed, prior studies have found that similar constructs, such as self-image and self-esteem, mediate or partially mediate associations between childhood maltreatment and later relationship violence (Reckdenwald et al., 2014; Goodman et al., 2021).

The current study advanced research on CEA and IPV by evaluating specific mechanisms underlying hypothesized pathways from CEA to later IPV perpetration and victimization. Multiple mediation analyses evaluated our hypothesis that compromised attachments to primary caregivers and decreased self-worth would account for predicted positive correlations between CEA and later IPV. All analyses held childhood IPV exposure constant to evaluate the specific contribution of CEA to later IPV, given prior research showing high rates of comorbidity between IPV exposure and CEA (Hamby et al., 2010). Finally, given that previous studies suggested sex-specific CEA effects (e.g., Richards et al., 2017), we also explored the proposed mediation model separately for females and males. The present study focused on a sample of college students because young adulthood coincides with establishing serious relationships.

METHOD

Participants & Procedures

Participants were 1,091 college students (68.4% female sex assigned at birth; Mage = 19.23, SD = 1.65) drawn from a broader study of young adult adaptation among 2,709 undergraduates attending a large public university in Southern California. Participants were included in the current study

if they reported having had a romantic relationship of two months or longer. The sample was racially and ethnically diverse (i.e., 41.9% Asian, 26.5% Latino, 15.9% White, 5.9% Black, and 9.9% multiracial/other).

Students were recruited from introductory psychology courses and individually completed a confidential online survey in private computer cubicles under the supervision of a trained examiner. Participants were compensated with course credit for their participation. The university's Institutional Review Board approved all procedures for Socio-Behavioral Research.

Measures

Maltreatment

The Childhood Maltreatment Interview Schedule (Briere, 1992) assessed CEA and childhood IPV exposure. This measure has demonstrated validity in predicting long-term outcomes of child maltreatment (Briere & Runtz, 1990). CEA was evaluated with 14 items inquiring about participants' experiences of being yelled at, humiliated, criticized, and insulted by each caregiver. Each item was rated on a 7-point scale, with 0 representing "never" and seven representing "more than 20 times" ($\alpha = 0.910$). The average score across caregivers was used to create a total CEA frequency variable for participants who rated two caregivers. Childhood IPV exposure included witnessing one caregiver physically assault the other as rated on a 7-point frequency variable, with one representing "once" and seven representing "more than 50 times."

Caregiver Attachment

Participants completed the Inventory of Parent and Peer Attachment to assess their attachment to their primary caregiver(s) (Armsden & Greenberg, 2009). Participants rated each caregiver on 17 items across three broad attachment domains: mutual trust, communication quality, and anger or alienation. Items were rated on a 5-point Likert scale, with one representing "almost never or never true" and 5 representing "almost always or always true" ($\alpha = 0.896$).

Global Self-Worth

Participants completed the Self-Perception Profile for College Students (Neeman & Harter, 1986). Global self-worth was indicated by six items asking students to select from two statements the one which best applied to them (e.g., "Some students are often disappointed with themselves" or "Other students are usually quite pleased with themselves), followed by a 2-point scale about how strongly they identified with the statement. A score of 1 represented a rating of "sort of true for me" and a 2 represented a rating of "really true of me" ($\alpha = 0.877$).

Young Adult Intimate Partner Violence (IPV)

Participants completed the Conflict Tactics Scale (Straus, 1979) to assess their experiences of IPV perpetration and victimization in a relationship of two months or longer. Items were rated for psychological aggression (e.g., swearing at a partner), physical assault (e.g., pushing a partner), and sexual coercion (e.g., making a partner have unprotected sex). IPV perpetration and victimization were indicated by 23 items each (αperpetration = 0.851; αvictimization = 0.830) using a 7-point frequency scale from 1 representing "one time" to 7 representing "21+ times."

Data Analytic Plan

All analyses were completed using IBM SPSS 28. Predictors and covariates were examined to ensure normality assumptions were met before conducting analyses. IPV perpetration and victimization scores were log-transformed to reduce kurtosis. A multivariate analysis of variance (MANOVA) with post hoc Bonferroni-corrected comparisons assessed mean differences across study variables by the sex assigned at birth, race/ethnicity, and their interaction. Correlation analyses evaluated associations between predictors, outcome variables, mediators, and

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covariates. Multiple mediation analyses were conducted using PROCESS version 4.2 (Hayes, 2022) to assess pathways from CEA to IPV through attachment and self-worth while controlling for participant sex assigned at birth and childhood IPV exposure.

RESULTS

Descriptive Statistics

A MANOVA revealed mean differences by the sex assigned at birth (Wilks' $\lambda = 2.757$, $p = 0.012$) and race/ethnicity (Wilks' $\lambda = 4.911$, $p < 0.001$), but not their interaction (Wilks' $\lambda = 1.393$, $p = 0.096$; see **Table 1**). Follow-up univariate ANOVAs showed that males reported stronger caregiver attachment, while females reported more IPV perpetration and victimization. There were significant differences across racial/ethnic groups regarding childhood IPV exposure, CEA, caregiver attachment, and self-worth. Participants who identified as multiracial/other reported more childhood IPV exposure than White and Asian participants. Asian and multiracial/other participants reported more CEA than White participants, while White and Latine participants had higher attachment scores than Asian and multiracial/other participants. Black and Latine participants reported higher self-worth than Asian participants, while White participants reported higher self-worth than Asian and multiracial/other participants.

	Participant Sex		Participant Race/Ethnicity				
	Female *M* (*SD*))	Male *M* (*SD*)	Asian *M* (*SD*)	Latine *M* (*SD*)	White *M* (*SD*)	Black *M* (*SD*)	Multi/other *M* (*SD*)
IPV exposure	.57 (1.34)	.31 (.84)	.38 (1.06)	.60 (1.32)	.28 (.90)	.64 (1.39)	.76[2,3] (1.45)
CEA	42.14 (20.19)	40.54 (19.89)	43.31[3] (21.01)	38.22 (18.38)	38.36 (18.15)	42.55 (19.21)	47.50[3] (20.68)
Caregiver Attachment	89.28[1] (20.34)	92.73 (17.15)	87.04 (19.10)	93.72[2,4] (18.62)	96.14[2,4] (19.57)	89.81 (17.16)	86.52 (20.69)
Self-worth	18.14 (4.38)	18.55 (3.96)	17.32 (4.07)	18.92[2] (4.38)	19.46[2,4] (4.11)	19.59[2] (4.12)	17.92 (4.08)
IPV Perpetration	3.42[1] (.31)	3.37 (.29)	3.41 (.31)	3.41 (.31)	3.39 (.26)	3.40 (.28)	3.43 (.34)
IPV Victimization	3.41[1] (.33)	3.38 (.32)	3.39 (.31)	3.40 (.31)	3.43 (.34)	3.35 (.29)	3.45 (.39)

CEA = Childhood Emotional Abuse. IPV = Intimate Partner Violence. [1]significant differences from males. [2]significant differences from Asians. [3]significant differences from White. [4]significant differences from multiracial/other.

Table 1. Descriptive Statistics for Study Variables by Participant Sex and Race/Ethnicity

Bivariate Statistics

CEA correlated positively with childhood IPV exposure, IPV perpetration, and IPV victimization and negatively with caregiver attachment and self-worth. Caregiver attachment and self-worth were positively correlated with IPV perpetration and negatively correlated with victimization. IPV perpetration and victimization were positively correlated.

Mediation Analyses

Controlling for participant sex assigned at birth and childhood IPV exposure, multiple mediation analyses showed significant indirect effects from CEA to IPV perpetration (**Figure 1**; $B = 0.0005$, $SE = 0.0002$, 95% CI [0.0002, 0.0009]) and victimization (**Figure 2;** $B = 0.0005$, $SE = 0.0002$, 95%

Variable	1	2	3	4	5	6
1. IPV exposure	—					
2. CEA	.22** .24** .14**	—				
3. Global self-worth	-.03 -.04 .02	-.30** -.30** -.29**	—			
4. Caregiver Attachment	-.22** -.24** -.12*	-.56** -.59** -.48**	.43** .44** .39**	—		
5. IPV Perpetration	.09** .08* .07	.21** .22** .19**	-.15** -.14** -.18**	-.12** -.09* -.13*	—	.
6. IPV Victimization	.06* .08* -.00	.17** .17** .17**	-.13** -.12** -.15**	-.12** -.10** -.11*	.82** .80** .87**	—

CEA = Childhood Emotional Abuse. IPV = Intimate Partner Violence. The top row shows the total sample, the middle row shows females, and the bottom row shows males. * $p < .05$. ** $p < .01$.

Table 2. Correlations for Total Sample (*N* = 1091), Females (*N* = 746) & Males (*N* = 345)

CI [0.0001, 0.0009]) through self-worth. Interestingly, when examined separately by sex assigned at birth, the indirect pathway from CEA to IPV perpetration was explained by both caregiver attachment (*B* = -0.0010, *SE* = 0.0004, 95% CI [-0.0018, -0.0001]) and self-worth for females (*B* = 0.0005, *SE* = 0.0002, 95% CI [0.0001, 0.0010]), but only by self-worth for males (*B* = 0.0005, *SE* = 0.0003, 95% CI [0.0000, 0.0012]). The indirect pathway from CEA to IPV victimization through self-worth was significant for females only (*B* = 0.0005, *SE* = 0.0002, 95% CI [0.0001, 0.0010).

DISCUSSION

The current findings highlight the implications of CEA and global self-worth for understanding pathways to IPV perpetration and victimization in young adulthood. Given that at least one in seven children in the United States experienced abuse or neglect between 2021 and 2022 (Center for Disease Control, 2022), as well as findings showing increased risk of adulthood violence among those with a history of abuse (e.g., Ehrensaft et al., 2003), this study provides a crucial and timely evaluation of mechanisms underlying the association between CEA and IPV in young adulthood. Consistent with prior research (e.g., Zurbriggen

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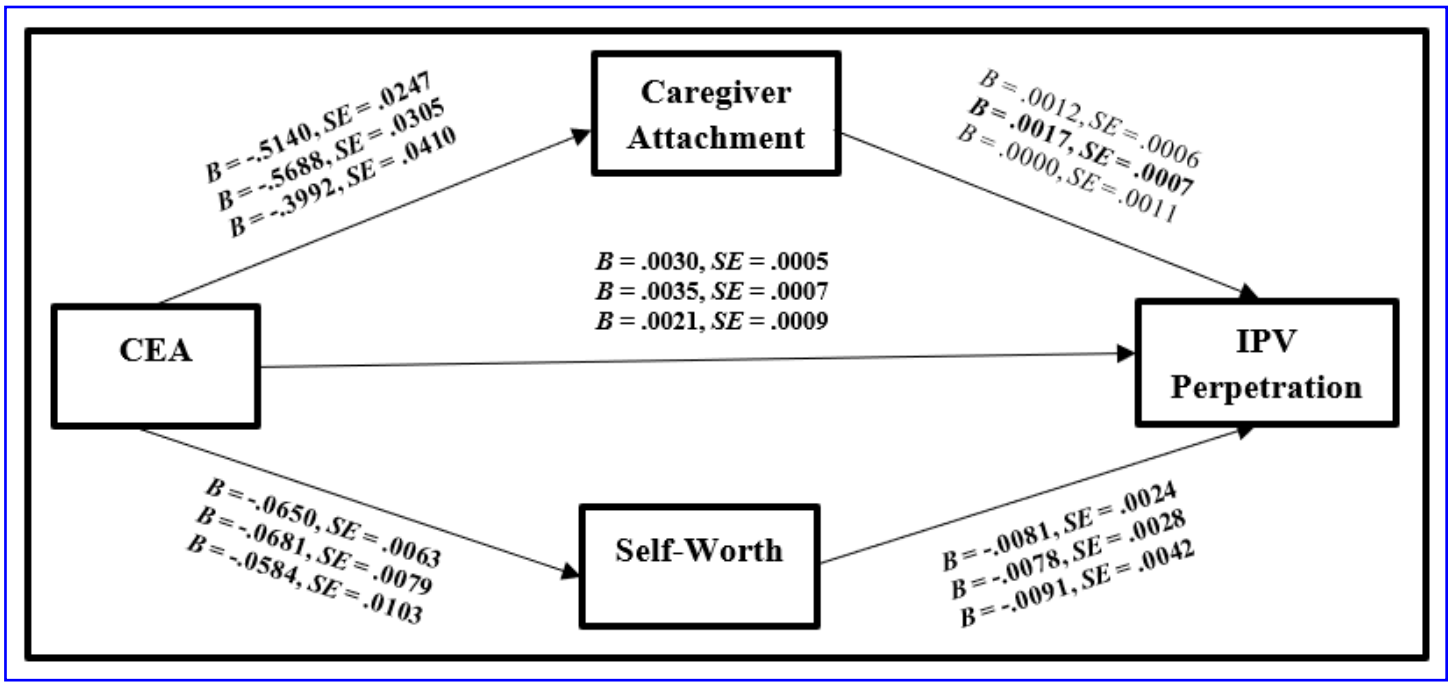

Figure 1. Regression of IPV perpetration on CEA through caregiver attachment and self-worth for the total sample (top), females (middle), and males (bottom). Bold denotes significant effects. Covariates (i.e., sex assigned at birth and childhood IPV exposure) are not shown for clarity.

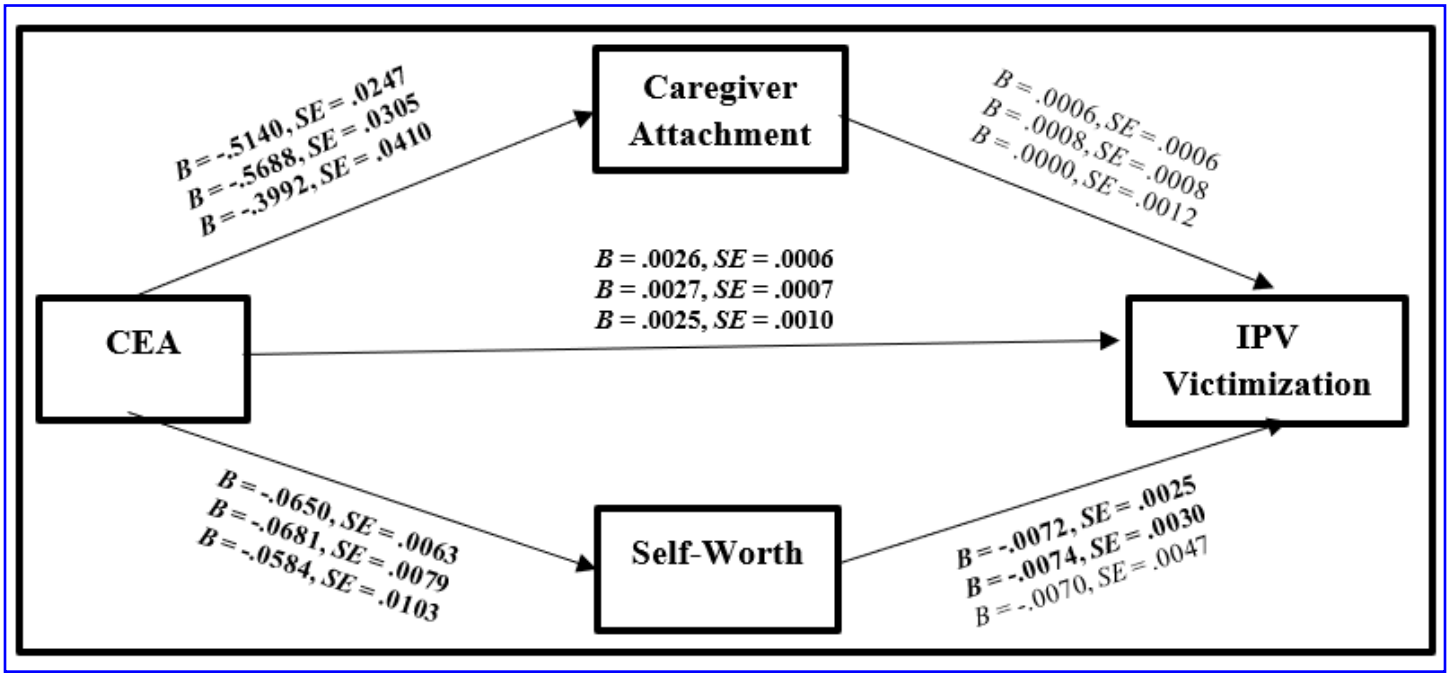

Figure 2. Regression of IPV victimization on CEA through caregiver attachment and self-worth for the total sample (top), females (middle), and males (bottom). Bold denotes significant effects. Covariates (i.e., sex assigned at birth and childhood IPV exposure) are not shown for clarity.

et al., 2010; Fosco et al., 2016; Richards et al., 2017), CEA was correlated with higher rates of IPV perpetration and victimization in young adulthood. Mediation analyses for the total sample revealed that, though CEA negatively affects caregiver attachment security and self-worth, only self-worth partially mediated relations between CEA and young adult IPV. Separated by the sex assigned at birth, the indirect pathway through self-worth to IPV perpetration was significant for both females and males, but the same indirect pathway to victimization was only significant for females. Interestingly, the adverse effects of CEA on caregiver attachment also explained IPV perpetration for females only. These findings, in part, contradict prior suggestions that CEA affects IPV for females only (Richards et al., 2017) while indicating female-specific pathways to IPV victimization through decreased self-worth and IPV perpetration through compromised caregiver attachments.

This investigation supports the cycle of violence hypothesis while extending this model to show that self-worth is an important and modifiable mediator of CEA effects on later IPV, particularly for females in the case of IPV victimization. These pathways are consistent with prior data showing self-esteem as a mechanism underlying the cycle of violence (e.g., Reckdenwald et al., 2014; Goodman et al., 2021). Although CEA contributed to poorer quality attachments, the non-significant pathway from attachment to IPV rendered the overarching pathway through attachment non-significant for both IPV perpetration and victimization in the total sample. However, caregiver attachment did play a significant explanatory role in predicting IPV perpetration for females.

Strengths & Limitations

This investigation offers a new look at explanatory pathways from CEA to IPV while holding childhood IPV exposure constant. Strengths of this project include the relatively large and demographically diverse sample to support these analyses. However, several limitations may have influenced the study findings while pointing to directions for growth in future research. First, this study used retrospective self-report measures of maltreatment. Multi-method maltreatment assessments can avoid confounds and missed information from individual measures (e.g., Zeanah & Humphreys, 2018; Grasso, 2022). Second, these data were collected 15 years ago and did not show representative numbers of participants with non-binary or transgender identities. Sexual minorities (e.g., lesbian, gay, etc.) were also underreported in the sample. Third, the cross-sectional nature of the study design limited our ability to draw causal inferences based on these findings. For example, it is equally plausible that IPV leads to lower self-worth as it is that it leads to IPV. Finally, the study drew on a convenience sample of college students, which can restrict the generalizability of the obtained findings to other young adult populations.

FUTURE RESEARCH RECOMMENDATIONS

The current study illuminates promising targets for future interventions to address and redress the adverse effects of CEA on later relational health. Future research should explore interventions aimed at improving self-worth among individuals with a history of CEA. There is a strong need to emphasize protective factors and positive developmental outcomes among maltreatment survivors and explore developmental problems. Many youths in this sample did not report later IPV, despite pronounced histories of CEA. Future research should identify ways to strengthen family relationships, improve self-worth, and mitigate the adverse effects of CEA on relational development. Moreover, although we could not evaluate the specific impacts of CEA across ethnic and racial groups in this sample, there is a pressing need for future studies to focus on within-group patterns rather than exclusively focusing on patterns between groups. Future research must replicate these findings using a broader probability sample (e.g., with young adults who are not in college and reside outside of Southern California).

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Together, these findings show that self-worth is an important mechanism underlying the cycle of violence from CEA to later IPV. Moreover, for females, caregiver attachments may also be a source of protection in pathways to IPV perpetration. The high prevalence of maltreatment in the US necessitates efforts to identify groups that are at greatest risk for adverse outcomes to guide the development and successful implementation of targeted, preventative interventions. At the same time, many participants in this study broke the cycle of violence, which points to a need for strength-based studies of resilience to inform prevention and intervention efforts further.

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Dressing Up the Revolution: The American Revolution in French Style, 1776-1789

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ABSTRACT

At the time of the American Revolution, French culture developed a fascination with America. This trend was expressed in many forms, including fashion, artwork, and architecture, all con-tributing to French narratives about the United States and the American Revolution. The fore-most of these narratives were the portrayals of America as a neoclassical, republican idyll and as a rustic, pastoral return to the romantic concept of nature. French figures who donned the "American" style played a large and often conscious role in crafting such cultural narratives. From 1776 to 1789, the "American" style in France took on a variety of contradictory meanings in French culture and politics. French figures like the Marquis de Lafayette, the Duchesse de la Tour du Pin, and the Marquis de Condorcet took on a French-American identity and styled themselves as "Americans" to express different political views. By analyzing these individuals' political views and sartorial styling, this essay examines the impact of French "American" style on the French perception of America. The essay utilizes contemporary publications such as newspapers and fashion magazines, as well as the memoirs and correspondence of French fig-ures. Through these sources, I examine the narratives surrounding "American" style and identi-ty in France. This reveals the complex relationship between French style and politics, contrib-uting to the ongoing historical discourse on the cultural lead-up to the Revolution of 1789.

KEYWORDS: American Revolution, France, fashion, 18th century

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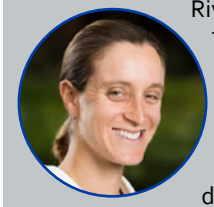

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Dressing Up the Revolution: The American Revolution in French Style, 1776-1789

INTRODUCTION

The American Revolution had a profound impact on the contemporary culture of France. French fascination with America was expressed in many forms of fashion, architecture, and artwork. Sources including fashion periodicals, political writings, and memoirs reflect this trend. One result of cultural exchange was the development of a popular "American" style in France, which encompassed a wide range of expressions, including clothing, hairstyles, and etiquette. Between 1776 and 1789, French expressions of American style and identity reflected French discourse surrounding America itself, shaping French narratives about the United States and the American Revolution. Different figures adopted American style to express different politics, as competing forms of sartorial presentation reflected changing pre-revolutionary political and philosophical currents. For example, the negligé, or informal style, of famous figures like Benjamin Franklin was recognized as a symbol of democracy and the American Revolution.[1] This trend was one facet of the American style popularized in French culture at this time.

THEMES OF AMERICAN STYLE

In France, American style was characterized by simplified dress and manners, which sought to evoke the rustic imagery of homespun farmers and Quakers.[2] This associated America with an idealized concept of "nature," which carried many meanings in contemporary France. In French discourse, popular works by Jean-Jacques Rousseau romanticized a return to a natural, uncorrupted state of politics, philosophy, and art. Following this theme, rustic fashions equated natural beauty to the natural virtues of civic society, promoting the ideal of a simple agrarian society, as opposed to absolute monarchy.[3] However, not all French writers agreed with this association. In the Tableau de Paris, a chronicle that described life in Paris during this time, French writer Louis-Sébastien Mercier called this fashion "affected simplicity," satirizing the affectations of elegants, the wealthy Parisians who followed the trend.[4] Mercier identified this trend as a reaction against the elaborate styles that had previously been popular in Paris.

Another theme that overlapped with American style was neoclassicism. Neoclassicism referenced Greco-Roman history, evoking concepts of democracy, republicanism, and "civic virtue,"[5] the meanings of which were often disputed in French discourse. This style, characterized by art, fashion, and architecture that evoked Antiquity, supported "classical models of liberty, political association, and artistic vitality." It emphasized an idealized image of "antique freedom" from the democratic civilizations of Ancient Greece and Rome.[6] In France, neoclassical elements of American style tied the United States to the legacy of ancient democracies and republics. This comparison turned America into an idealized example of democracy and representative government, further associating the United States and the American Revolution with the political philosophy of the Enlightenment.

Both the neoclassicism and the idealized rusticity of

1 Kimberly Chrisman-Campbell, "American Idols: Fashions 'à l'américaine' in Prerevolutionary France." *Transactions of the American Philosophical Society* 109, no. 5 (2021): 190. http://www.jstor.org/stable/45381471.

2 Robert Darnton, *George Washington's False Teeth: An Unconventional Guide to the Eighteenth Century* (New York, NY: W.W. Norton & Company, 2003): 128.

3 Dagmar Freist, "Belief," in *A Cultural History of Dress and Fashion in the Age of Enlightenment*, ed. Peter McNeil, vol. 4 (Bloomsbury Academic, 2021): 104.

4 Mercier Louis-Sébastien, *Panorama of Paris: Selections from Le Tableau De Paris*, trans. Jeremy D. Popkin (University Park, PA: Pennsylvania State University Press, 2003): 61.

5 Freist, 104.

6 Amelia Rauser, *The Age of Undress: Art, Fashion, and the Classical Ideal in the 1790s* (New Haven, CT: Yale, 2020): 189.

American style associated the United States with a utopian image of equality. Writers like Michel Guillaume Jean de Crèvecœur, a French aristocrat who traveled to America and published The Letters of an American Farmer, described the United States as a nation "close to nature," where "the first principles of universal morality" arose naturally from the citizens' simple, agriculture-based lifestyle.[7] While these idealized portrayals did not go undisputed, they were extremely popular in France, shaping French perceptions of America and fueling contemporary discourse.

The French discourse surrounding American style also suggests that, across the French social order, many people were aware of its political meanings. Fashion periodicals such as the Galeries des Modes, which ran from 1778 to 1787,[8] were published primarily in Paris, the center of French fashion. These publications were read by people of all social classes, especially women.[9] Fashion magazines established a "continuous visual present" by informing readers of constantly changing trends, typically set by the upper classes.[10] In addition to providing realistic illustrations,[11] these publications also explained the meaning of each fashion. This context situated trends like American style within the intense political discourse of France, which was discussed, through France's rich print culture, across different social classes.

One of the most famous and controversial examples of an American fashion trend was the pouf coiffure. Poufs were elaborate headpieces worn over women's tall hairstyles. This style was worn almost exclusively during the period of the American Revolution and often reflected French opinions toward America.[12] Poufs were highly politicized hairstyles, often referencing key events or topics of debate in France. During the American Revolution, the Galerie des Modes published illustrations of styles entitled "Bunker's Hill, or America's Headdress"[13] and "Independence, or The Triumph of Liberty,"[14] accompanied by text with news of events like the Battle of Bunker Hill and naval victories over the English. Clothing was also used to convey "American" sentiments. Another fashion plate, published in 1779, shows a full outfit labeled habit à l'insurgent, or "dress of the insurgents," with a description praising American women for their role in the American Revolution.[15] The Tableau de Paris mentions similar styles, named "The Boston" and "The Philadelphia", both of which expressed support for the American Revolution.[16]

The Tableau de Paris also addresses the political nature of this style, mocking the Parisians who dressed with a "republican air" without understanding the "serious questions" of contemporary politics, particularly the discourse surrounding England and the United States.[17]

7 Darnton, 124.

8 Stella Blum, *Eighteenth-Century French Fashion Plates: 64 Engravings from the "Galerie Des Modes,"* 1778-1787 (New York, NY: Dover Publications, 2016): 2.

9 Christian Huck, "Visual Representations," in *A Cultural History of Dress and Fashion in the Age of Enlightenment*, ed. Peter McNeil, vol. 4 (Bloomsbury Academic, 2021): 183.

10 Huck, 180.

11 Huck, 180.

12 Kimberly Chrisman-Campbell, *Fashion Victims: Dress at the Court of Louis XVI and Marie-Antoinette* (New Haven: Yale University Press, 2015): 170.

13 Lynn Festa and Joseph Roach, "Fashion and Adornment," in *Cultural History of Hair in the Age of Enlightenment*, ed. Margaret K. Powell, vol. 4 (Bloomsbury Academic, 2022): 69.

14 Chrisman-Campbell, 161.

15 Chrisman-Campbell, 184.

16 Mercier, 65.

17 Mercier, 148.

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Mercier's comments reflect French interest in British-American politics, satirizing French people who took on Anglo-American fashions without understanding the political ramifications of the style. This also demonstrates the widespread popularity of American style, which was worn throughout Paris, the center of French culture.

However, in many cases, French concepts of American style deviated from the fashions that were worn in contemporary America. For example, after the American Revolution, many women in Philadelphia began to wear elaborate "English" fashions, including silk clothing and "borrowed hair," or hairpieces.[18] This fashion, which broke with the idealized French concept of "American" dress, provoked a negative reaction from Brissot de Warville, a visiting French aristocrat. Brissot viewed elaborate, "European" style clothing as a detriment to the United States' civic virtue, as it conflicted with the rustic ideal of a simple, agriculture-driven republic.[19] This shows how French "American" style shaped French perceptions of actual American people, particularly women, who were expected to uphold the neoclassical virtues of the United States' republic.

Similarly, conflicted sentiments are reflected in broader French discourse surrounding the United States. The "craze for America" in France went much deeper than aesthetic style, extending to the topics of politics and philosophy.[20] During this period, many French individuals cultivated "American" identities, a role that had multiple controversial meanings. The conflicts between different French individuals' American identities became increasingly apparent as political discourse deepened in the lead-up to the French Revolution.

FRENCH "AMERICANS"

The Marquis de Lafayette, a French aristocrat who fought in the American Revolution and supported the American cause in France, styled himself as an American and gained American citizenship during this time. Lafayette's expressions of American style often played into contemporary neoclassical aesthetics, evoking an idealized image of Greco-Roman culture.[21] Throughout the 1780s, as Lafayette constructed and decorated a new household in Paris, he deliberately mixed American and neoclassical elements. The facade of this building was based on Greco-Roman designs. Lafayette also hired the furniture designer Bernard Moliter, known for neoclassical design, and displayed his "American mementos" along with Greco-Roman symbols.[22] Lafayette associated this style with "the virtues of ancient republics," comparing the United States to the Republic of Ancient Rome.[23]

Another French individual who took on both an American style and identity was Henriette-Lucie Dillon, Marquise de la Tour du Pin. Like many other educated, aristocratic Frenchwomen, Tour du Pin engaged with politics and philosophy through salons, where she met influential American figures like Benjamin Franklin and Thomas Jefferson. Her husband, the Marquis de la Tour du Pin, also served as a military officer in the American Revolution.[24] Tour du Pin supported her husband's political goals as he sought to apply the "American experience" of Enlightenment

18 Kate Haulman, *The Politics of Fashion in Eighteenth-Century America* (Chapel Hill, NC: University of North Carolina Press, 2011): 194.
19 Haulman, 195.
20 Darnton, 121.
21 Laura Aurrichio, *The Marquis: Lafayette Reconsidered* (Vintage Books, 2014): 134.
22 Aurrichio, 134.
23 Aurrichio, 134.
24 Caroline Moorehead, *Dancing to the Precipice: Lucie De La Tour Du Pin and the French Revolution* (London: Vintage, 2010): 71.

to France,[25] cultivating an "American" style in their household to reflect these ideas. Tour du Pin embraced the "newfound taste for simplicity in dress" and directly compared her style to that of American women.[26]

In her memoirs, Tour du Pin describes her American style and eventual move to America as a rustic, idealized escape from French culture and society. She compares the United States to Ancient Rome,[27] evoking a neoclassical, utopian view of America, somewhat similar to Lafayette's writings. However, Tour du Pin directly opposed Lafayette's view of America, referring to him as a "simpleton" and accusing him of misrepresenting the American Revolution through his supposedly "insipid," inaccurate memoirs.[28] This reflects their polarized views on the French absolute monarchy. Following Lafayette's participation in conflicts like the 1787 Assembly of Notables, Tour du Pin accused him of using American style and rhetoric to support radical politics. This demonstrates how French individuals who adopted the American style often came into conflict over what American ideals represented in France.

The Marquis de Condorcet provides a contrasting example of the conflicts inherent to French "American" identity. During this period, Condorcet published multiple political writings about America, including a pamphlet entitled The Influence of the American Revolution on Europe, published in 1788. In this pamphlet, Condorcet claimed that the American Revolution reflected the "progress of the Enlightenment," referencing Rousseau's concepts of uncorrupted nature and the "common will" of each nation's people.[29] Through his work, Condorcet supported the political and philosophical ideas associated with America, identifying himself as an "American" philosopher.[30]

Condorcet also expressed this support through personal style, as he appears to have deliberately donned "Americanized" clothing for portraits like a painting done by the artist Jean-Baptiste-François Bosio. This fashion is similar to the style worn by both Lafayette and Tour du Pin, evoking a rustic version of American style. Condorcet references this view in his writing, referring to the United States as a "nation of farmers."[31] Despite this shared sartorial style, Condorcet's writings came into conflict with other French "Americans," who wrote idealistically of America. Condorcet challenged aristocratic French writers like Brissot de Warville and Crèvecœur, both of whom used America as a "utopian" idyll in their works.[32] In response to these exaggerated portrayals of America, Condorcet provided a more realistic interpretation of the American Revolution centered on the "concept of progress."[33] Condorcet sought to praise the progress achieved by the United States while also acknowledging injustices like the American institution of slavery. His direct challenge to other French writers shows how the meanings of American style and identity were frequently and publicly disputed in France.

Discourse over America and American identity reflected the current political conflicts in France. By adopting American style and supporting the American cause, French

25 Moorehead, 85.

26 Moorehead, 66.

27 Lucie Henriette de la Tour du Pin, *Memoirs of Madame de la Tour du Pin*, trans. by Felice Harcourt (New York, NY: McCall, 1971): 283.

28 Tour du Pin, 273.

29 Durand Echeverria, "Condorcet's The Influence of the American Revolution on Europe." *The William and Mary Quarterly* 25, no. 1 (1968): 101. https://doi.org/10.2307/1920807.

30 Darnton, 121.

31 Echeverria, 104.

32 Darnton, 128.

33 Darnton, 122.

individuals of various political persuasions could espouse support for their vision of the Enlightenment. These visions ranged from the monarchist opinions of Tour du Pin, who supported reform through France's absolute monarchy, to the revolutionary ideas of writers like Condorcet, who supported the republican concepts of the United States' newly formed government. The contradictory "American fantasies" portrayed in France, often at odds with the reality of the United States,[34] reflect the issues that divided French politics at this time.

During the intense political upheaval that occurred in France between 1776 and 1789, many French individuals disputed the definition and enactment of the politics and philosophy of the Enlightenment. Many challenged the authority of the French absolute monarchy, using America as a symbol of Enlightened government. In this context, American style and identities provided an area of discourse, where French individuals disputed their conflicting visions for the future of France. Individuals who donned this style, particularly political writers like Condorcet, were often highly conscious of the conflicting and controversial ideas that it represented.

For example, during the 1788 Assembly of Notables, while some French "Americans" supported the concepts of democracy and republicanism, others advocated for more conservative approaches to reform, particularly the implementation of a constitutional monarchy. One conflict occurred when Lafayette sided with a faction of monarchist Breton nobles. This caused Condorcet, an ally of Lafayette, to express concern that Lafayette had given up his Enlightenment ideals for "noble prerogatives."[35] In a friendly condemnation of Lafayette's political actions, Condorcet referenced the Potomac and the Continental Army of America, alluding to both Lafayette's and his identification with America. Despite their shared "American" identity, the tension between Lafayette and Condorcet's different political ideas was increasingly apparent in 1788. This reflects growing pre-revolutionary conflict and division over the future of the French monarchy.

CONCLUSION

After 1789, the conflicting nature of different French-American identities became far more evident and controversial. During the French Revolution, many French individuals who had previously shared an American style and identity found themselves in opposing political factions. After the Flight to Varennes in 1791, Lafayette continued to argue in favor of a constitutional monarchy, while Condorcet supported republicanism, eventually allying himself with the Girondin faction.[36] In her memoirs, Tour du Pin strongly opposed the views of both Lafayette and Condorcet, finding their interpretations of the American Revolution to be too radically republican and revolutionary.[37] Despite their alliance during the 1770s and 1780s, the conflict of the French Revolution revealed that these three French "Americans" held very different political views, and had donned American style in support of their respective political factions. Some supported radical republicanism, while others advocated only for conservative reforms to the monarchy. The different factions of French "Americans" demonstrate the close and complex relationship between style and politics during this period of intense political conflict.

In future research, historians should draw from overlooked sources to better understand the different factions that arose during this complex period of French history. Fashion periodicals and personal documents, including memoirs and correspondence, could be used to examine politics from a

34 Darnton, 121.

35 Auricchio, 167.

36 David Williams, *Condorcet and Modernity* (Cambridge: Cambridge University Press, 2007): 33.

37 Tour du Pin, 273.

new angle. For example, analyzing the appropriation of rustic and neoclassical imagery in the French Revolution may yield new, complex interpretations of this controversial period. By examining how different individuals and movements styled themselves, future research can delve into sociopolitical history from a fresh perspective.

From 1776 to 1789, American style in France took on many different forms and meanings. The natural and neoclassical themes that defined this style associated the United States with the ideas of the Enlightenment, shaping French narratives about America and the American Revolution. French styling effectively "dressed up" the American Revolution, providing an area of discourse for many conflicting views of this event. French individuals who donned this style and cultivated "American" identities did so in support of vastly different politics, ranging from absolute monarchy to republicanism. In the tumultuous, changing world inhabited by French figures like Lafayette, Tour du Pin, and Condorcet, the American Revolution had major, controversial, and far-reaching effects. The styles and identities through which these French "Americans" expressed themselves are powerful windows into the complex politics and philosophy of their time.

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Three Classical Theorems on Interchanging Limits With Integrals in Calculus

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ABSTRACT

The interchange of the 'limit of an integral' with the 'integral of a limit' for sequences of functions is crucial in relevant applications, such as Fourier series for decomposing periodic functions into sinusoidal components, and Fubini's theorem for changing the order of integration of multivariable functions. This expository paper reviews three classical results in real analysis for cases where the limit of an integral of a sequence of functions equals the integral of the limiting function: (1) Monotone Convergence Theorem, (2) Uniform Convergence Theorem, and the broadest result, (3) Dominated Convergence Theorem. While proofs of (2) are typically studied in undergraduate analysis, the proofs of (1) and (3) are usually reserved for graduate-level measure theory, where they are taught in a more general context. The purpose of this paper is to summarize and adapt W. A. J. Luxembourg's undergraduate-friendly proof [7] of (3) Arzel`a's Dominated Convergence Theorem, to demonstrate the nontrivial direction of (1) Monotone Convergence Theorem for Riemann Integrals. Our aim is to demystify the hidden logic involved in these well-established theorems, making them more accessible for undergraduate analysis.

KEYWORDS: Real Analysis, Riemann Integrals, Arzel`a's Dominated Convergence Theorem, Monotone Convergence Theorem, Uniform Convergence Theorem

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Sudhir Murthy is a fourth-year Mathematics major. He is interested in the niche intersection of category theory, type theory, and logic. Under the guidance of Dr. Estela Gavosto and Dr. Michael Nelson, he has presented a paper on this topic at the Fourth Annual Undergraduate Philosophy Conference, hosted by the University of Texas, Dallas. As a recipient of Khan Academy's First Talent Search Award in 2015, he aspires to earn a Ph.D. in Mathematics and hopes to become a professor.

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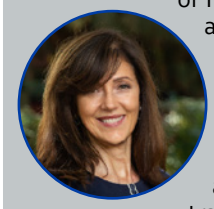

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Three Classical Theorems on Interchanging Limits

Introduction

The conditions for when a limit may be interchanged with an integral played a crucial role in the development of Fourier Series and Real Analysis. Consider the heat equation $\frac{\partial u}{\partial t} = \frac{\partial^2 t}{\partial^2 x^2}$ subject to the constraints $u(0,t) = u(1,t)$ for all t and $u(x,0) = f(x)$ for some "well-behaved" function f. Here, $u(x,t)$ represents the temperature of a circular rod of unit length at position $x \in [0,1]$ and time $t \geq 0$. Fourier's novel solution to this equation, which was awarded the Académie des Sciences Grand Prix [5], is

$$u(x,t) = \sum_{n=1}^{\infty} \left(2 \int_0^1 f(x') \sin(n\pi x')\, dx' \right) \sin(n\pi x) e^{-n^2\pi^2 t}.$$

However, as shown by Cauchy [2] and discussed by Dieudonné [4], rigorously verifying the proposed solution involves exchanging the order of limits with integrals.

As another example, consider the well-known argument justifying the series expansion of $\log(1+x)$. For a real number $|t| < 1$, the geometric series $\sum_{n=0}^{\infty}(-t)^n$ converges to $\frac{1}{1+t}$. Integrating from 0 to x, where $|x| < 1$, yields:

$$\int_0^x \frac{1}{1+t}\, dt = \int_0^x \sum_{n=0}^{\infty} (-t)^n\, dt \tag{1.1}$$

$$\implies \log(1+x) = \int_0^x \lim_{k\to\infty} \sum_{n=0}^{k} (-t)^k\, dt \tag{1.2}$$

$$\implies \log(1+x) = \lim_{k\to\infty} \int_0^x \sum_{n=0}^{k} (-t)^n\, dt \tag{1.3}$$

$$\implies \log(1+x) = \lim_{k\to\infty} \sum_{n=0}^{k} \int_0^x (-t)^n\, dt \tag{1.4}$$

$$\implies \log(1+x) = \sum_{n=0}^{\infty} \frac{(-1)^n x^{n+1}}{n+1}. \tag{1.5}$$

The seemingly intuitive, but unjustified, step in this derivation is the interchange between the integral and the limit in (1.3).

This paper provides an overview of three conditions for when we may interchange limits with integrals of sequences of functions: (**1**) a monotone sequence of functions, (**2**) a uniformly converging sequence of functions, and (**3**) the weakest

condition, a dominated sequence of functions. First, we shall recall some basic definitions. We consider the bounded closed interval $[a, b]$ for real numbers $a < b$. We use $\mathbb{N}$ to denote the set of natural numbers and $\mathbb{R}$ to denote the set of real numbers.

Definition 1.1. A sequence of functions $f_n : [a, b] \to \mathbb{R}$ is said to be:

(i) *Monotone* if and only if either $f_{n+1}(x) \geq f_n(x)$ for every $x \in [a, b]$ and $n \in \mathbb{N}$ (*monotone increasing*), or $f_n(x) \geq f_{n+1}(x)$ for every $x \in [a, b]$ and $n \in \mathbb{N}$ (*monotone decreasing*).

(ii) *Uniformly convergent* if and only if there exists some $f: [a, b] \to \mathbb{R}$ such that $f_n \to f$ pointwise, and for every $\varepsilon > 0$ there exists an $N \in \mathbb{N}$ such that $|f_n(x) - f(x)| < \varepsilon$ for all $x \in [a, b]$ and $n \geq N$.

(iii) *Dominated* if and only if there exists a positive, real M such that $|f_n(x)| \leq M$ for every $x \in [a, b]$.

One could weaken conditions (**1**) and (**3**) to "uniformly monotone" and "uniformly bounded," conveying the notions of sequences of functions that eventually behave in a monotone or bounded manner, that is, for all sufficiently large indices. The advantage of such an approach is the insight that conditions for when a limit may be interchanged with an integral require some form of "uniformity." The cost, however, is lengthier proofs. For example, proofs involving the limiting behavior of uniformly monotone sequences are mostly the same for monotone sequences, but with the added step of taking a sufficiently large m-tail subsequence to ensure monotonicity. By acknowledging uniformity here, we hope to provide the reader with this insight while maintaining the simplicity of the original conditions.

In section two, we review the Uniform Convergence Theorem, usually taught as an exercise in undergraduate analysis, and the easy direction of the Monotone Convergence Theorem. In section three, we follow Luxemburg's proof [8] of the Dominated Convergence Theorem intended for undergraduate-level analysis. In the fourth section, we adapt his ideas to present a proof of the challenging direction of the Monotone Convergence Theorem. Finally, in the conclusion, we highlight the importance of understanding the logic underlying these proofs and their utility for computer-verified mathematics. Although the digital formalization of real analysis has largely been achieved, it seems to lack undergraduate-friendly proofs of the Dominated Convergence Theorem for Riemann Integrals. This paper aims to take the first steps towards bridging this gap by elucidating the logic underpinning these paper-pen proofs.

Three Classical Theorems on Interchanging Limits

Integrals of Monotone and Uniformly Convergent Sequences

We partially prove the result that limits can be interchanged with integrals for monotone sequences. The full result borrows the set-up in Luxemburg's paper. We prove the easy direction for now and save the other as Theorem 4.1.

Our paper is based on Darboux integrals defined as upper and lower rectangular estimates over partitions of $[a, b]$ by taking suprema and infima. By convention $\mathcal{B}[a, b]$, $\mathbb{R}[a, b]$, and $\mathcal{C}[a, b]$ represent, respectively, the family of bounded, Riemann integrable, and continuous real-valued functions on $[a, b]$.

Theorem 2.1 (Monotone Convergence Theorem, Part 1). *Let* $f, f_n \in \mathcal{R}[a, b]$ *with* $f_n \to f$ *pointwise and suppose* f_n *is monotone increasing. Then, the sequence* $\int_a^b f_n$ *converges and, moreover,*

$$\lim_{n\to\infty} \int_a^b f_n \leq \int_a^b f.$$

Proof. Since $f_n(x) \leq f_{n+1}(x)$ for all $x \in [a, b]$ and $n \in \mathbb{N}$, by monotony of integration,

$$\forall n \in \mathbb{N} \quad \int_a^b f_n \leq \int_a^b f_{n+1}.$$

It follows from induction

$$\forall n \in \mathbb{N} \quad \int_a^b f_n \leq \int_a^b f,$$

and, consequently, the result follows. □

Example 2.1, by Bradley [1], provides a remarkable application of this theorem to produce a family of non-trivial results.

Example 2.1. For $f \in \mathcal{R}[0, 1]$ nonnegative, we show

$$\lim_{n\to\infty} \int_0^1 n \log\left(1 + \frac{f}{n}\right) \leq \int_0^1 f.$$

Note that the sequence of functions $n \log\left(1 + {}^{f}/_{n}\right) = \log\left(1 + {}^{f}/_{n}\right)^{n}$ converges to $\log(e^{f}) = f$. Moreover, since f is nonnegative, the sequence $(1 + f/n)^{n}$ is monotone increasing. As the logarithm is an increasing function, it follows that $\log\left(1 + {}^{f}/_{n}\right)^{n}$ is also monotone increasing, and thus Theorem 2.1 applies. Indeed, after proving Theorem 4.1, we can strengthen this example with equality.

We now turn our attention to the classical result found in calculus textbooks: the limit can be interchanged with an integral under uniform convergence.

Theorem 2.2 (Uniform Convergence Theorem). *Let* $f_n \in R[a,b]$ *converge uniformly to* $f\colon [a,b] \to \mathbb{R}$. *Then* $f \in \mathcal{R}[a,b]$ *and, moreover,*

$$\lim_{n\to\infty} \int_a^b f_n = \int_a^b f.$$

Proof. Fix $\varepsilon > 0$ and choose $N \in \mathbb{N}$ such that $|f_n(x) - f(x)| < \frac{\varepsilon}{(b-a)}$ for all x and $n \geq N$. In particular, $f_N(x) - \frac{\varepsilon}{(b-a)} < f(x) < f_N(x) + \frac{\varepsilon}{(b-a)}$ for all x, and as f_N is bounded it follows f is bounded. Thus, f possesses lower and upper integrals, and

$$(i)\ \forall n \geq N \quad \overline{\int_a^b} f - \int_a^b \frac{\varepsilon}{b-a} \leq \int_a^b f_n \leq \overline{\int_a^b} f + \int_a^b \frac{\varepsilon}{b-a}, \text{ and}$$

$$(ii)\ \forall n \geq N \quad \underline{\int_a^b} f - \int_a^b \frac{\varepsilon}{b-a} \leq \int_a^b f_n \leq \underline{\int_a^b} f + \int_a^b \frac{\varepsilon}{b-a}.$$

As $\varepsilon > 0$ is arbitrary, $\int_a^b f_n \to \overline{\int_a^b} f$ and $\int_a^b f_n \to \underline{\int_a^b} f$, so f is Riemann integrable on $[a,b]$ and $\int_a^b f_n$ converges to the common value $\int_a^b f$.

Pointwise convergence is not a strong enough condition to justify the interchange of limit with integral, as shown by **Example 2.2**.

Example 2.2. Define the sequence of functions on [0, 1] by $f_1(x) = 1$ and

$$\forall n \geq 2 \quad f_n(x) := \begin{cases} n^2 x & \text{if } 0 \leq x < 1/n \\ 2n - n^2 x & \text{if } 1/n \leq x < 2/n \\ 0 & \text{otherwise.} \end{cases}$$

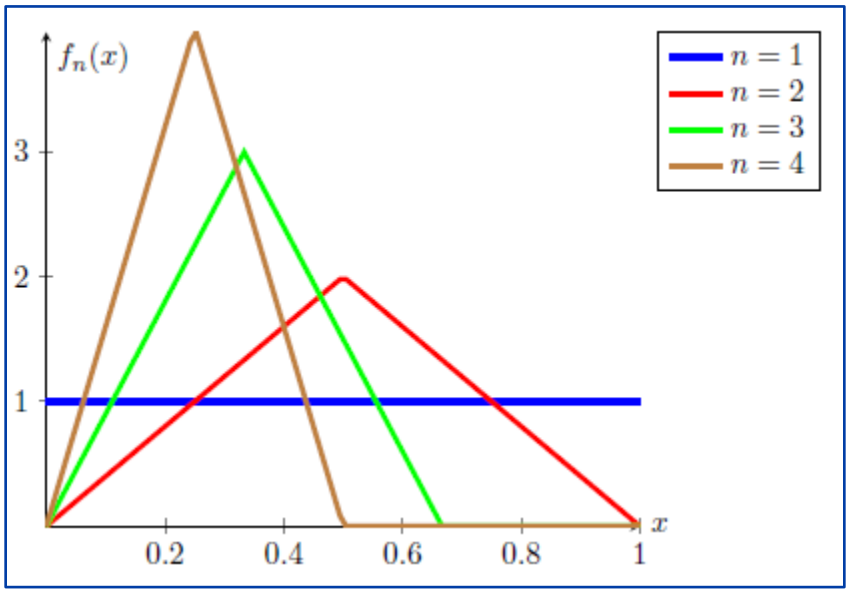

FIGURE 2.1 Graphs of the first four terms of the sequence of functions $f_n(x)$.

As illustrated by **Figure 2.1**, each function represents an isosceles triangle with height n and base $2/n$ and, thus, has unit area. However, f_n converges pointwise to the zero function and, thus, we have the disagreement $\lim_{n\to\infty} \int_0^1 f_n \neq \int_0^1 f$.

ARZELÀ'S DOMINATED CONVERGENCE THEOREM

Uniform convergence is a sufficient condition for interchanging a limit with an integral; however, Dominated Convergence Theorem weakens the condition. We follow W. A. J. Luxemburg's proof [8] of Arzelà's Dominated Convergence Theorem. We set the stage for his main arguments by proving Lemmas 3.1 and 3.2.

Lemma 3.1 (Dini's Uniform Convergence Theorem). *Let* $g_n, g \in C[a,b]$ *where* $g_n \to g$ *pointwise and* g_n *is monotone. Then the convergence is uniform.*

Proof. It is enough to show a monotone decreasing sequence $f_n \in C[a,b]$ that converges pointwise to 0, converges uniformly. We can transform the general problem $g_n \to g$ by putting $f_n \coloneqq g_n - g$ or $f_n \coloneqq g - g_n$, whichever ensures the sequence of functions f_n monotonically decreases.

Given $\varepsilon > 0$, first define $N\colon [a,b] \to \mathbb{N}$ and then $\delta\colon [a,b] \to \mathbb{R}$ by:

$$N(x) := \min\{N' \in \mathbb{N} \mid \forall n \geq N',\ |f_n(x)| < \varepsilon/2\}\ \text{, and}$$

$$\delta(x) := \sup\{\delta' \in [0, b-a] \mid \forall t \in \mathcal{B}_{\delta'}(x) \cap [a,b],\ |f_{N(x)}(t) - f_{N(x)}(x)| < \varepsilon/2\}.$$

Observe that $N(x)$ is well-defined since the pointwise convergence of $f_n(x)$ guarantees the corresponding set of natural numbers is nonempty. Using continuity of $f_{N(x)}$ at x guarantees $\delta(x)$ is well-defined by completeness, since the corresponding set of real numbers is bounded and nonempty. In fact, this supremum is also an element of the set and, thus, is the maximum.

For each $x \in [a,b]$, since $\{f_n\}_{n\in\mathbb{N}}$ is decreasing and nonnegative,

$$(1) \qquad |f_n(t)| \leq |f_{N(x)}(t)| \leq |f_{N(x)}(t) - f_{N(x)}(x)| + |f_{N(x)}(x)| < \varepsilon$$

for every $n \geq N(x)$ and $t \in \mathcal{B}_{\delta(x)}(x) \cap [a,b]$. The idea is the family of open intervals $\{\mathcal{B}_{\delta(x)}(x)\colon x \in [a,b]\}$ is an open cover of $[a,b]$, and thus by compactness contains a finite subcover, say $\{\mathcal{B}_{\delta(x_1)}(x_1), \ldots, \mathcal{B}_{\delta(x_n)}(x_n)\}$ for some $n \in \mathbb{N}$ and $x_1, \ldots, x_n \in [a,b]$. By taking $N \coloneqq \max\{N(x_1), \ldots N(x_n)\}$, we can solve our current ε, N criterion as shown by **Figure 3.1**.

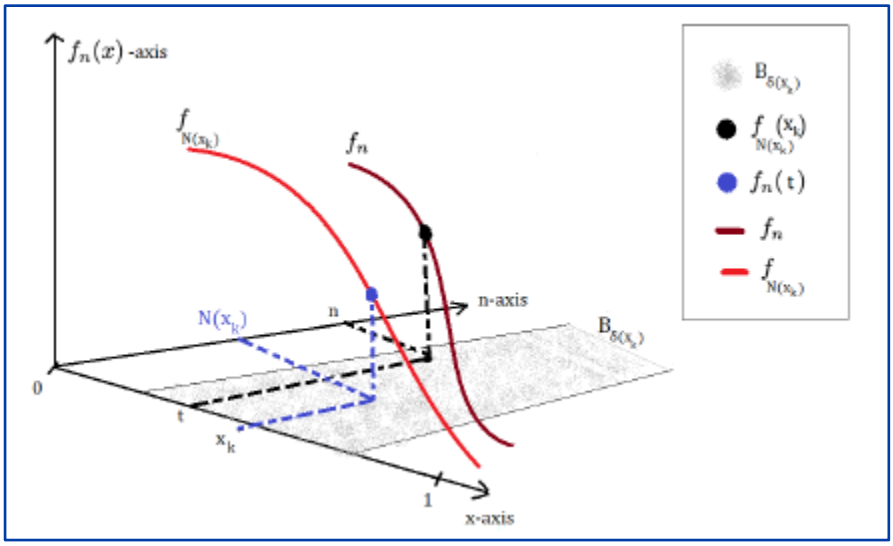

FIGURE 3.1 Any $t \in [a, b]$ belongs to an open interval $\mathcal{B}_{\delta(x_k)}(x_k)$ for some $k \in N$. Since any $n \geq N$ is greater than or equal to $N(x_k)$, we can use an instance of (1) to estimate $|f_n(t)| < \varepsilon$.

Remark. We explicitly construct $N(x)$ and $\delta(x)$, and, therefore, avoid accidental applications of the axiom of choice. Notice our comment that the supremum is the maximum in the definition of $\delta(x)$ is not superficial. It is precisely this topological property we use in our proof.

The condition $g \in C[a, b]$ in the theorem is needed. For example, consider $g_n(x) := x^n$ defined on [0, 1]. The sequence monotonically decreases to the discontinuous function $g(x) := 1$ if $x = 1$ and $g(x) := 0$ otherwise. The convergence, however, is not uniform.

Lemma 3.2. *For all nonnegative* $f \in \mathcal{B}[a, b]$ *and* $\varepsilon > 0$, *there exists* $g \in C[a, b]$ *such that* $0 \leq g(x) \leq f(x)$ *for all* $x \in [a, b]$ *and*

$$\underline{\int_a^b} f - \varepsilon \leq \int_a^b g.$$

Proof. Fix nonnegative $f \in \mathcal{B}[a, b]$ and $\varepsilon > 0$. By definition of lower integral, there must be a partition $P := \{x_0, \ldots, x_n\}$ of $[a, b]$, where $a = x_0 < \cdots < x_n = b$ and

$$\underline{\int_a^b} f - \varepsilon/2 < L(P, f).$$

Using this partition, define a step function s on $[a, b]$ by

$$s(x) := \inf_{t \in [x_{i-1}, x_i]} f(t)$$

where $[x_{i-1}, x_i]$ is the first interval from the partition which contains x.

By design, $0 \leq s(x) \leq f(x)$ and $\int_a^b s = L(P, f)$. We can define g as a continuous extension of s by connecting lines between its steps (see Figure 3.2) to ensure:

(1) $0 \leq g(x) \leq s(x)$ for every $x \in [a, b]$, by adjusting the points for which g breaks into a line, and

(2) the magnitude of the integral underneath the lines is in total less than $\varepsilon/2$ by adjusting their widths to be as small as needed.

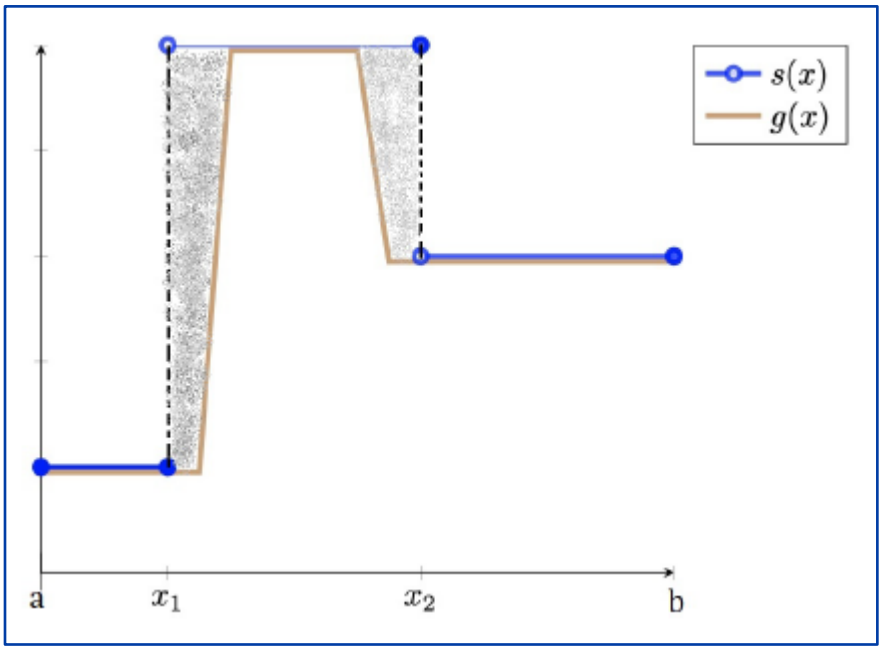

FIGURE 3.2. The blue graph $s(x)$ represents the step function and the brown graph $g(x)$ is its continuous approximation as used in the proof of Lemma 3.2. Since there are finitely many jumps, we can make the total shaded area smaller than $\varepsilon/2$ while preserving the inequality $0 \leq g(x) \leq f(x)$ for all $x \in [a, b]$.

This is possible since the lines are bounded and have at most $n + 1$ steps. Thus $0 \leq g(x) \leq s(x) \leq f(x)$ and

$$\underline{\int_a^b} f - \varepsilon < \int_a^b s - \varepsilon/2 < \int_a^b g.$$

Three Classical Theorems on Interchanging Limits

The stage is set; we are ready for Luxemburg's main proof from [8].

Theorem 3.1. (Dominated Convergence Theorem). *Let* $f_n, f \in \mathcal{R}[a,b]$ *where* $f_n \to f$ *pointwise and* f_n *is dominated. Then*

$$\lim_{n\to\infty} \int_a^b f_n = \int_a^b f.$$

Proof. Without loss of generality, we may assume $f_n \to 0$ pointwise and that there is some $M \in \mathbb{R}$ such that $0 \leq f_n(x) \leq M$ for all $x \in [a,b]$. We can always redefine a new sequence of functions $|f_n - f|$, and apply the implication

$$\lim_{n\to\infty} \int_a^b |f_n - f| = 0 \implies \lim_{n\to\infty} \int_a^b f_n = \int_a^b f$$

to recover a proof for the original problem.

Set $p_n(x) := \sup_{k\geq n} f_k(x)$ for all $x \in [a,b]$ and $n \in \mathbb{N}$; note this is well-defined since for $x \in [a,b]$ fixed, the sequence $f_n(x)$ is bounded by M. Moreover, observe that p_n monotonically decreases to 0 and $0 \leq f_n(x) \leq p_n(x)$ for all x and n. The key technique used in this proof, which we will show in the next section, involves constructing a sequence of continuous functions h_n such that:

(1) $\forall x \in [a,b]\ \forall n \in \mathbb{N} \quad 0 \leq h_n(x) \leq p_n(x),$

(2) $\forall n \in \mathbb{N} \quad \underline{\int_a^b} p_n - \varepsilon \leq \int_a^b h_n,$ and

(3) h_n monotonically decreases to 0.

Note that p_n is lower integrable because it is bounded by M, so condition (2) is sensible. While (1) and (2) are clearly implied by Lemma 3.2, the "magic step" is (3). We reveal in the next section how Luxemburg constructs such a sequence.

By Lemma 3.1, we conclude that $\int_a^b h_n = 0$. Since

$$0 \leq \int_a^b f_n \leq \underline{\int_a^b} p_n \leq \int_a^b h_n + \varepsilon,$$

in the limit we have

$$0 \leq \lim_{n\to\infty} \int_a^b f_n \leq \varepsilon.$$

As $\varepsilon > 0$ is arbitrary, $\lim_{n\to\infty} \int_a^b f_n = 0$ and our proof is complete.

This theorem provides an efficient means for computing limits of integrals.

Example 3.1. Consider $\lim_{n\to\infty} \int_0^1 \sin(x^2/n)\, dx$. Without the theorem, we would struggle to find a closed-form anti-derivative. But it is easy to check the sequence of functions $\sin(x^2/n)$ is dominated and converges pointwise to 0. Thus, the limit computes to 0.

Full Monotone Convergence Theorem for Integrals

We return to the study of monotone sequences and aim to extend Theorem 2.1 to the full Monotone Convergence Theorem. Our proof adapts Luxemburg's justification of the "magic step" employed in Theorem 3.1.

Theorem 4.1 (Monotone Convergence for Riemann Integrals Part 2). *Let* $f, f_n \in \mathcal{R}[a,b]$ *with* $f_n \to f$ *pointwise and suppose* f_n *is monotone decreasing. Then*

$$\lim_{n\to\infty} \int_a^b f_n \leq \int_a^b f.$$

Proof. Without loss of generality, we may assume f monotonically decreases to 0 and, therefore, is nonnegative. We can redefine a new sequence of functions by $f_n \to f$, which monotonically decreases to 0 and show instead $\lim_{n\to\infty} \int_a^b f_n \leq 0$.

Fix $\varepsilon > 0$. Its is enough to construct a sequence $h_n \in C[a,b]$ such that:

(1) $\forall x \in [a,b]\ \forall n \in \mathbb{N} \quad 0 \leq h_n(x) \leq f_n(x)$,

(2) $\forall n \in \mathbb{N} \quad \underline{\int_a^b} f_n - \varepsilon \leq \int_a^b h_n$, and

(3) h_n monotonically decreases to 0.

For once constructed, by Lemma 3.1 it follows $\lim_{n\to\infty} \int_a^b h_n = 0$, and, therefore, $\lim_{n\to\infty} \int_a^b f_n \leq \varepsilon$. As ε > 0 is arbitrary, the proof is finished.

By Lemma 3.2, we form a sequence $g_n \in C[a,b]$ such that $0 \leq g_n \leq f_n$ and $\int_a^b f_n - \varepsilon/2^n \leq \int_a^b g_n$ for all n. Now for each n, define $h_n := \min\{g_1,\ldots,g_n\}$. It is easy to verify that h_n is a sequence of continuous functions which satisfies properties (1) and (3); the challenge is demonstrating (2).

It is clear that $\underline{\int_a^b} f_1 - \varepsilon \leq \int_a^b h_1$, so it remains to show $\underline{\int_a^b} f_n - \varepsilon \leq \int_a^b h_n$ for $n \geq 2$. From h_n being defined as a minimum, it follows

$$0 \leq g_n \leq h_n + \sum_{k=1}^{n-1} (\max\{g_k,\ldots,g_n\} - g_k) \leq h_n + \sum_{k=1}^{n-1} (f_k - g_k).$$

Note that the final inequality comes from $g_k \leq f_k$ for all k and the sequence f_k monotonically decreasing. So, by taking the lower integral, we have

$$\begin{aligned}
\int_a^b g_n &\leq \int_a^b h_n + \underline{\int_a^b} \left(\sum_{k=1}^{n-1} f_k - g_k \right) \\
&\leq \int_a^b h_n + \sum_{k=1}^{n-1} \left(\underline{\int_a^b} f_k - \int_a^b g_k \right) \\
&\leq \int_a^b h_n + \sum_{k=1}^{n-1} \frac{\varepsilon}{2^k}.
\end{aligned}$$

Since $\underline{\int_a^b} f_n - \varepsilon/2^n \leq \int_a^b g_n$, it follows

$$\underline{\int_a^b} f_n \leq \int_a^b g_n + \varepsilon/2^n \leq \int_a^b h_n + \sum_{k=1}^{n} \frac{\varepsilon}{2^k} \leq \int_a^b h_n + \varepsilon,$$

as desired.

Remark. While the process of generating g_n suggests the use of axiom of countable choice, we can avoid it. By reinserting the mechanism of how each g was obtained in the proof of Lemma 3.2, we can constructively generate the sequence g_n. This method of obtaining h_n, by taking the minimum of $g_1,\ldots,g_n$ is the "magic step" omitted earlier in Theorem 3.1.

Both parts of monotone convergence, i.e. Theorems 2.1 and 4.1, prove Monotone Convergence Theorem for integrals in general.

Corollary 4.1 (Monotone Convergence Theorem). *Let f,* $f_n \in \mathcal{R}[a,b]$ *with* $f_n \to f$ *pointwise and* f_n *monotone. Then*

$$\lim_{n\to\infty} \int_a^b f_n = \int_a^b f.$$

Proof. When f_n is monotonically increasing, then $-f_n$ is monotonically decreasing so Theorem 4.1 proves the missing inequality

$$\lim_{n\to\infty} \int_a^b f_n \geq \int_a^b f.$$

Similarly, when f_n is monotonically decreasing, Theorem 2.1 proves

$$\lim_{n\to\infty} \int_a^b f_n \geq \int_a^b f.$$

Corollary 4.2 is an interesting generalization of this result for sequences of integrable functions which need not fully converge pointwise.

Corollary 4.2 (Fatou's Lemma). *Let* $f_n \in \mathcal{R}[a,b]$ *and suppose, for* $x \in [a,b]$ *fixed, the sequence* $f_n(x)$ *is bounded so that limit superiors exist. Given* $\lim_{n\to\infty} \sup f_n \in \mathcal{R}[a,b]$ *and* $\sup_{k\geq n} f_k \in \mathcal{R}[a,b]$*, then*

$$\lim_{n\to\infty} \sup \int_a^b f_n \leq \int_a^b \lim_{n\to\infty} \sup f_n.$$

Proof. Observe that $\sup_{k\geq n} f_k$ is a decreasing sequence of functions and converges pointwise to $\lim_{n\to\infty} \sup f_n$. Thus

$$\lim_{n\to\infty} \sup \int_a^b f_n \leq \lim_{n\to\infty} \int_a^b \sup_{k\geq n} f_k = \int_a^b \lim_{n\to\infty} \sup f_n,$$

where the equality comes from Theorem 4.1 and the inequality comes from the observation that $f_n \leq \sup_{k\geq n} f_k$ for all n.

The inequality in Fatou's Lemma cannot be strengthened.

Example 4.1. Define a sequence of functions on [0, 1] by

$$f_n(x) := \begin{cases} (-1)^n & \text{if } x < 1/2 \\ (-1)^{n+1} & \text{otherwise.} \end{cases}$$

Observe that all integrals vanish, i.e. $\int_0^1 f_n = 0$ for all n. However, for fixed n, the suprema $\sup_{x\in[a.b]} f_n(x) = 1$.

Thus $\lim_{n\to\infty} \sup \int_a^b f_n = 0$ and $\int_0^1 \lim_{n\to\infty} \sup f_n = 1$.

Conclusion

Understanding when a limit and integral commute is important for grasping the scope of various techniques in calculus. We have re-proven three classical theorems on the interplay of limits with integrals: (**1**) Monotone Convergence Theorem, (**2**) Uniform Convergence Theorem, and (**3**) Dominated Convergence Theorem. While (**2**) was a familiar exercise, we have shown how the methods used by Luxemburg's proof of (**3**) can be adapted to prove (**1**). We have explicitly discussed the hidden logic underlying these proofs in the hopes of demystifying them and making them more accessible to undergraduate students in real analysis. Our goal, albeit modest, is to help strengthen foundations and foster enthusiasm for more advanced topics in measure theory.

A benefit of demystification is its application to the formalization of mathematics through digital proof systems such as Lean [6]. Founded on Thierry Coquand's pioneering work [3] on the calculus of constructions, Lean is an interactive theorem prover for mathematicians to write and check proofs. Through the improvements of AI technologies and proof automation, we believe computer-verified mathematics will become more mainstream in the near future. By presenting proofs written closer to the axiomatic level, computer scientists, mathematicians, and logicians may find it easier to transport them into Lean. Our paper is a humble attempt in this direction.

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