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University of California, Riverside

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Undergraduate Research Journal

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From the Administration



As a research university, one of UC Riverside's most important duties is the creation of knowledge. From exploring the inner workings of the mind to examining the cultural impacts of science fiction literature, UCR is at the forefront of discovery. The *UC Riverside Undergraduate Research Journal*, now in its 12th volume, epitomizes UCR's commitment to ensuring that students at all levels are a part of this effort.

Undergraduate research is a hallmark of UCR's scholarly and educational missions. 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 joy of scholarly research. As you will see in this 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. I congratulate all the students who contributed to this edition of the Journal, and I express my sincere gratitude to the faculty mentors that supported these students in their research journeys.

I hope you enjoy this edition of the *UCR Undergraduate Research Journal*.



Kim W. Wilcox
Chancellor



Innovation, Creativity, and Scholarship; these ideals, which are the hallmarks of any institution of higher education, are readily expressed in a variety of ways at UCR. Since 2007, this campus has proudly supported the publication of the UCR Undergraduate Research Journal, a rigorously reviewed volume, that publishes the highest quality examples original work by our students. Since research is seldom an individual effort, I personally thank those who have mentored the contributors to this volume including faculty members, graduate students, postdoctoral fellows, undergraduate peers, and staff who have passionately supported these students through this journey. I am especially grateful to the contributors themselves who have committed themselves to the high standards that original research demands and have disseminated their work in this journal. It is my hope that these

experiences will not only encourage you to continue as a lifelong explorer but that your work will ignite a spark in future generations of students at UCR.

A critical component to producing any quality research journal is the substantial effort that goes into the peer review process. In this regard, I would like to thank the Student Editorial Board, the Faculty Advisory Board, and especially Gladis Herrera-Berkowitz for their critical and professional efforts in producing this outstanding volume that serves as a testament to the highest standards of excellence.

Sincerely,



Richard A. Cardullo, Ph.D.
Interim Vice Provost, Undergraduate Education
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It is with great pleasure to present UC Riverside's Twelfth Edition of the Undergraduate Research Journal. It has been a truly rewarding experience to work with dedicated individuals, all of whom have contributed to the success and advancement of undergraduate research and legacy of the Journal itself. Congratulations to all the authors for your excellence – your achievements are a hallmark to UCR's academic and creative culture, built upon the pursuit of knowledge. Congratulations also to the Student Editorial Board and Faculty Advisory Board – your dedication and diligence to the publication process have assured the quality and success of the Journal. I am extremely grateful to have been a part of this team of talented individuals and to have contributed to the journey that is the Undergraduate Research Journal.

Kollan Doan — Editor-In-Chief



Back (left to right) – Daniel Choi, Nolan Winicki, Jordan Cohen, Amrik Kang, Nicole Huffman, Debora Handoyo

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Since UCR's *Undergraduate Research Journal* started, it has published almost 130 scholarly articles across many fields. These papers represent the commitment of our undergraduates to performing independent research as part of their experience at UCR. The Journal thus fills a critical need for our students. More often than not, undergraduate research forms part of a larger work with many contributors, which can mean a dilution of the student's contributions as well as a longer wait time between completion of the work and its publication. With the *Undergraduate Research Journal*, our students can write about their specific research findings and get first-author credit. They can publish before the end of their degree, and gain the experience of seeing their manuscript go through a peer-review and publication process just like articles in a standard research journal. When a paper becomes a part of a student's professional experience, it contributes to their record of scholarly achievement in a unique way. The submission and review process is run primarily by undergraduates who form the Student Editorial Board, working with members of the Faculty Advisory Board. We owe a debt of gratitude to the students for their professionalism and dedication for the review and preparation of the articles you see here. We are also grateful for the participation of the members of the Faculty Advisory Board in guiding the student reviewers. If you are interested in publishing your undergraduate research at UCR, consider submitting to our next issue!

A handwritten signature in blue ink that reads "Morris F. Maduro".

Dr. Morris F. Maduro
Chair of the Undergraduate Research Journal Faculty Advisory Board
Professor of Biology

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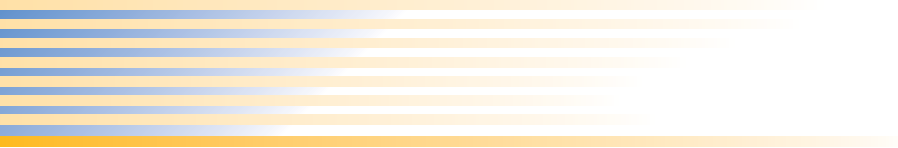
*Department of Art
& Department of Media
and Cultural Studies*

Caylin Yorba-Ruiz is a third-year double major in Art and Media & Cultural Studies. Her work utilizes art to advocate for issues regarding mental health, feminism, environmentalism, and sexual violence awareness. Through funding from the Healthy Campus Initiative, Caylin is working on self-care coloring books and free survivor-directed zines for the UCR community. Currently, she interns at CARE, is the Marketing Assistant for Undergraduate Admissions, and is a Unit Assistant for the ARC. Caylin plans on pursuing a PhD in media studies.



“Untitled” by Caylin Yorba-Ruiz

This photo was taken at the UCR Botanic Gardens of green moss on a branch. It was inspired by a photography assignment given through a course offered by the Department of Art. The course prompted students to create intriguing work in which perspective was utilized. As an artist, I am most inspired by simplicities and small workings within the world, such as the intricate structure of flowers and the process of pollination. I believe these workings and simplicities are reflections of faith, hope, resilience, and love. The beauty of the world motivates and inspires me to continue my work in social justice, ecology, and mental health. I created this image in hopes that it will act as a means to impel individuals to be more cognizant of nature, even the small self-sustaining systems pictured in this image. Moreover, I chose this image as it illustrates my desire to create work that embodies and articulates issues that are cross disciplinary — something I believe that all good work does. I hope that this image inspires individuals across disciplines to view art as a medium which can string various disciplines together and thereby cultivate solidarity amongst those disciplines.



A Subset of Brain Neurons Controls a Sexually Dimorphic Proboscis Holding Behavior in Adult *Drosophila Melanogaster*

Sameera Ahmad¹, Kush Amin¹, Yu-Chieh David Chen², Ryan Joseph¹, and Anupama Dahanukar¹

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Sameera Ahmad

Department of Molecular, Cell and Systems Biology

Sameera Ahmad is a third year Biology major. She has studied the relationship between potential gustatory neurons and feeding behaviors in vinegar flies for two years under Dr. Anupama Dahanukar. With funding from the National Science Foundation and Highlander Excellence Scholarship, she served as a peer mentor in Dr. Dahanukar's research program this past summer. A former intern at Dr. Esther Ahn Optometry, she plans to become an optometrist while branching into eye research.

Kush Amin

Department of Molecular, Cell and Systems Biology

Kush Amin is a third-year Biology major. His research explores the link between potential gustatory neurons and feeding behaviors in vinegar flies. He also served as a peer-mentor for Dr. Dahanukar's research program this past summer funded by the National Science Foundation (NSF). Additionally, he is a Quality Management Coordinator for the COPE Health Scholars program at Riverside Community Hospital. In the future, Kush plans to pursue a career in the field of medicine.

ABSTRACT

Taste is essential for humans and animals alike to evaluate food quality and make important decisions about food choice and intake. How complex brains process sensory information to produce behavior is an essential question in the field of sensory neurobiology. Currently, little is known about taste circuits in the brain as compared to other sensory systems. Here, we used the common vinegar fly, *Drosophila melanogaster*, to explore the potential role of brain neurons labeled by a transgenic line (*VT041723-GAL4*) in producing "proboscis holding" behavior (extrusion of the mouthpart without withdrawal). By utilizing the *GAL4/UAS* binary expression system, we expressed a heat-activated cation channel (*UAS-dTrpA1*) in these brain neurons and artificially activated them by elevation of temperature, subsequently examining behavior in the heat-activated proboscis extension reflex (PER) assay. We found that activation of these neurons induced proboscis holding. Interestingly, the proboscis holding phenotype was sexually dimorphic. Male flies rarely showed proboscis holding and those that did had shorter proboscis holding durations. On the other hand, both mated and virgin females showed significantly more proboscis holding and had longer proboscis holding durations than male flies. Overall, we identified a subset of brain neurons labeled by the *VT041723-GAL4* line that controls a sexually dimorphic feeding response (proboscis holding) upon activation.

KEYWORDS: *Drosophila melanogaster*; GAL4/UAS system; taste circuit; feeding behavior; proboscis extension; sexual dimorphism



FACULTY MENTOR

Dr. Anupama Dahanukar

Associate Professor in the Department of Molecular, Cell and Systems Biology

Dr. Anupama Dahanukar is an Associate Professor in the Department of Molecular, Cell and Systems Biology. She received her PhD in Genetics at Duke University. Dr. Dahanukar completed postdoctoral studies at Yale University and joined the faculty of UC Riverside in 2009. Her research focuses on understanding mechanisms that underlie various aspects of taste recognition and feeding behaviors in *Drosophila melanogaster* and other insects using a multi-disciplinary approach spanning molecular genetics, high-throughput genomics, electrophysiology, imaging and behavior analysis.

INTRODUCTION

One of the fundamental questions in the field of neuroscience is how the human brain responds to different sensory inputs. However, the human brain is too complex to study directly. Therefore, to address this fundamental question, we take advantage of a powerful genetic model organism, *Drosophila melanogaster*; better known as the common vinegar fly. There are several advantages of using the vinegar fly as an experimental model. They have numerically simpler brains, and they are genetically much simpler than mammals with only four pairs of chromosomes, allowing for easier manipulation. They also have fewer neurons compared to their mammalian counterparts, which can be specifically pinpointed with genetic tools. They are easy to rear in a simple cornmeal-based diet and they have a short life cycle of about ten days, allowing researchers to carry out experiments with ease. Notably, vinegar flies have sensory systems similar to those of humans, thus they still exhibit complex behaviors seen in humans, such as mating or feeding (Vosshall and Stocker, 2007). Therefore, the fundamental principles of how neuronal circuits in the fly brain process sensory information have the potential to be applied to human neurobiology as well.

The gustatory system of the fruit fly has emerged as a powerful model to explore the molecular and cellular basis of taste, due to the identification of chemosensory receptor genes and development of methods to assess feeding behaviors. In addition, the molecular genetic approaches available in flies allow us to activate or silence specific neurons. This enables us to explore the functional roles of specific neurons in feeding behaviors. Although there are many studies on the role of peripheral taste neurons in sensing various chemicals (Chen and Dahanukar, 2017; Ling et al., 2014; Raad et al., 2016; Weiss et al., 2011), little is known about how the peripheral sensory information is processed by higher-order neurons in the brain. In this study, we aim to use the *Drosophila* gustatory system to study candidate higher-order brain neurons that might be involved in processing taste information and mediating feeding behaviors.

To address this question, we used the Vienna Tile *GAL4* (*VT-GAL4*) transgenic fly lines combined with the *GAL4/UAS* binary expression system to access different subsets

of neurons in the adult fly brain (Kvon et al., 2014). We expressed heat-activated ion channels (*dTrpA1*) in different subsets of neurons labeled by different *VT-GAL4* lines (Kang et al., 2011) and examined the effects of neuronal heat activation on the extrusion of the proboscis, which is the elongated mouthpart of the fly involved in feeding. The extension behavior is commonly known as the proboscis extension reflex (PER) (Shiraiwa and Carlson, 2007). Our data suggests that one of the candidate *VT-GAL4* lines (*VT041723-GAL4*), which activates a unique proboscis holding response in the heat-activated PER assay, may be involved in processing taste information.

MATERIALS AND METHODS

Fly strains

Flies were reared on standard cornmeal-dextrose-agar food at 25°C and 60-70% relative humidity under a 12 hour:12 hour dark:light cycle. Three transgenic fly strains were used in this study: *VT041723-GAL4* (Vienna Drosophila Resource Center) (Kvon et al., 2014), *UAS-GFP* (Weiss et al., 2011), and *UAS-dTrpA1* (Bloomington Drosophila Stock Center #26263). The genotype of the experimental fly line was *UAS-dTrpA1/UAS-dTrpA1; VT041723-GAL4/VT041723-GAL4*. We had two control genotypes: the *VT-GAL4* only control (+/+; *VT041723-GAL4/VT041723-GAL4*) and the *UAS-dTrpA1* only control (*UAS-dTrpA1/UAS-dTrpA1; +/+*). The +/+ chromosomes indicate wild-type. With only one transgene each, these control genotypes did not have *GAL4/UAS* binary expression and were expected to show no proboscis extensions. Thus, the control flies also ensured that observed proboscis extensions in experimental flies resulted from the binary expression of the *GAL4* and *UAS* transgenes, rather than either of the transgenes alone.

Heat-activated proboscis extension reflex (PER) assay

Individual flies were immobilized on glass coverslips with drops of clear, non-toxic nail polish (Sally Hansen Insta-Dri Top Coat) and then incubated for 1-2 hours in a humidified chamber made by filling a pipette tip box with water and placing damp Kimwipes (Kimberly-Clark Kimtech) on top. One by one, each coverslip containing an individual fly was placed on a 34°C heat block and proboscis extension behaviors were observed under a light microscope. We recorded the following parameters for

each experimental trial: trial number, sex, participation, and extension duration. In all experiments, we tested male, mated female, and virgin female flies. To eliminate other variables, we kept the ages and sample size of flies for each sex constant.

Statistical analyses

All data are presented as mean \pm S.E.M. Statistical tests were conducted using Prism 7 (GraphPad Software). Differences between means of different groups were evaluated for statistical significance with the Kruskal-Wallis Test, followed by the *post hoc* Dunn's multiple comparison test.

RESULTS

To identify higher-order neurons in the brain that process taste information, we took advantage of the transgenic resources in the Vienna Tiles GAL4 (*VT-GAL4*) Library at the Vienna Drosophila Resource Center (VDRC). GAL4 is a yeast transcriptional activator that can bind to *UAS* sequences, thereby inducing gene expression downstream of the *UAS* sequences. In the *VT-GAL4* library, 964 *VT-GAL4* lines with different genomic DNA sequences show different labeling patterns that can be visualized by genetic crosses with *UAS-GFP* transgenic flies. The expression patterns of these *VT-GAL4* lines in the adult *Drosophila* brain have been well-documented on the VDRC website (Kvon et al., 2014). The Dahanukar lab has done a preliminary image-based screen for neurons that arborize in and around the subesophageal zone (SEZ), the primary taste center in the fly brain, and pinpointed several potential candidate lines. In this study, one candidate was selected for further analysis: *VT041723-GAL4* (*VT23-GAL4*). The *VT23-GAL4* line labels neurons in the anterior SEZ as well as other taste areas of the fly brain, suggesting that these neurons might be involved in processing taste information (**Figure 1A**).

To determine whether the *VT23*-labeled neurons are involved in feeding behaviors, we artificially activated these neurons and measured proboscis extension. We expressed the *Drosophila* transient receptor potential channel, subfamily A, member 1 (*dTrpA1*), a heat-activated cation channel (Kang et al., 2011), in *VT23*-labeled neurons through the *GAL4/UAS* binary expression system

(Brand and Perrimon, 1993). By crossing *UAS-dTrpA1* transgenic flies with *VT23-GAL4* transgenic flies, we are able to use the progeny from the crosses and activate the *VT23*-labeled neurons by elevating ambient temperature to 34°C. We used the proboscis extension reflex (PER) as a feeding behavioral readout (Shiraiwa and Carlson, 2007). Proboscis extension has been extensively used as a robust feeding behavior assay where the fly protrudes its mouthpart (proboscis) as an indication of food acceptance when sensing attractive chemicals in the environment. Interestingly, we found that activation of *VT23*-labeled neurons caused a unique PER response in which flies do not retract the proboscis but keep it extended for several minutes or longer. We therefore have termed this behavior “proboscis holding” (**Figure 1B**).

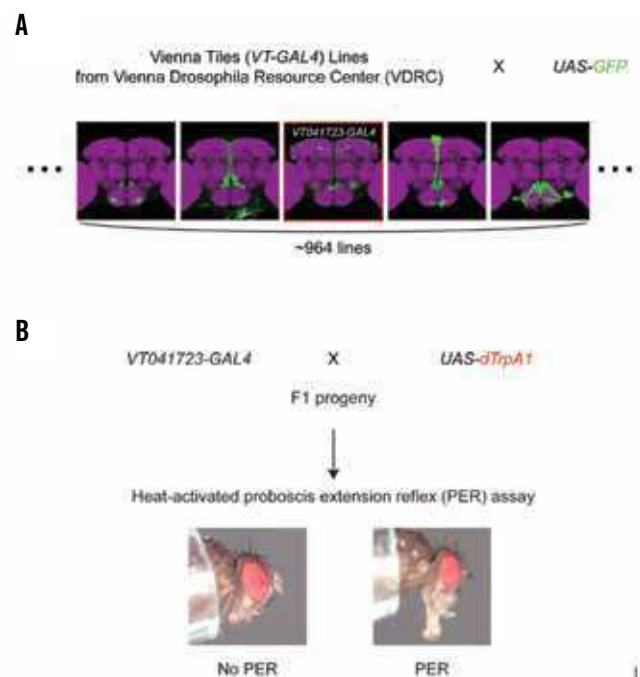


Figure 1. A feeding behavior screen of the *VT-GAL4* transgenic library from the VDRC identifies *VT041723-GAL4* labeled neurons as a potential candidate for higher-order taste neurons.

A. Green Fluorescent Protein (GFP) expression patterns in the *Drosophila* brain driven by the 964 Vienna Tiles *GAL4* (*VT-GAL4*) lines are documented in the Vienna Drosophila Resource Center (VDRC). Five different sets of brain neurons labeled by *VT-GAL4* lines are shown as examples. The red box in the middle indicates the *VT-GAL4* line (*VT041723-GAL4*) that was further analyzed based on a previous screen done in the lab.

B. Activation of *VT041723-GAL4* labeled neurons is achieved by expression of *dTrpA1*, a heat-activated ion channel. Extensions of the fly mouthpart (proboscis) are scored by the heat-activated proboscis extension reflex (PER) assay.

To determine if both males and females exhibited the same proboscis holding upon activating *VT23*-labeled neurons, we performed the heat-activated PER assay with male, mated female, and virgin female flies for both experimental and control genotypes. The proboscis holding phenotype was recorded on an all-or-nothing basis. If a fly had an extension time for one minute or longer upon heat activation, it was counted as proboscis holding with a value of 1. If there was no extension or the extension was shorter than one minute, it was given a value of 0. As expected, the *VT23-GAL4* and *UAS-dTrpA1* controls of all three test conditions did not show any proboscis holding. The experimental *VT23>dTrpA1* flies demonstrate varying levels of proboscis holding across sex and mating status. We found that 12.7% of male flies, 60.5% of mated female flies, and 33.3% of virgin female flies showed the proboscis holding response (N = 150; N = 172; N = 45, **Figure 2A**). Mated female flies had greater participation than both virgin female and male flies, while virgin females had

greater participation than the male flies. In summary, we found that activation of *VT23*-labeled neurons in the adult fly brain induces proboscis holding and such behavior may be sexually dimorphic, since female flies show significantly greater participation than male flies.

To further investigate the nature of proboscis holding in *VT23>dTrpA1* flies, we also recorded the duration of proboscis holding. We analyzed the average time of proboscis holding only for *VT23>dTrpA1* flies, since the controls flies did not show this behavior. We timed the flies for a maximum of 420 seconds due to time restraints. We found that the average proboscis holding durations were 119.7±27.01 seconds for male flies, 244.1±15.54 seconds for mated female flies, and 393.4±26.58 seconds for virgin female flies (N = 19; N = 104; N = 10, **Figure 2B**). The virgin female flies had a significantly longer time of proboscis holding than mated females and males. In addition, female flies had a significantly longer time of proboscis holding than male flies.

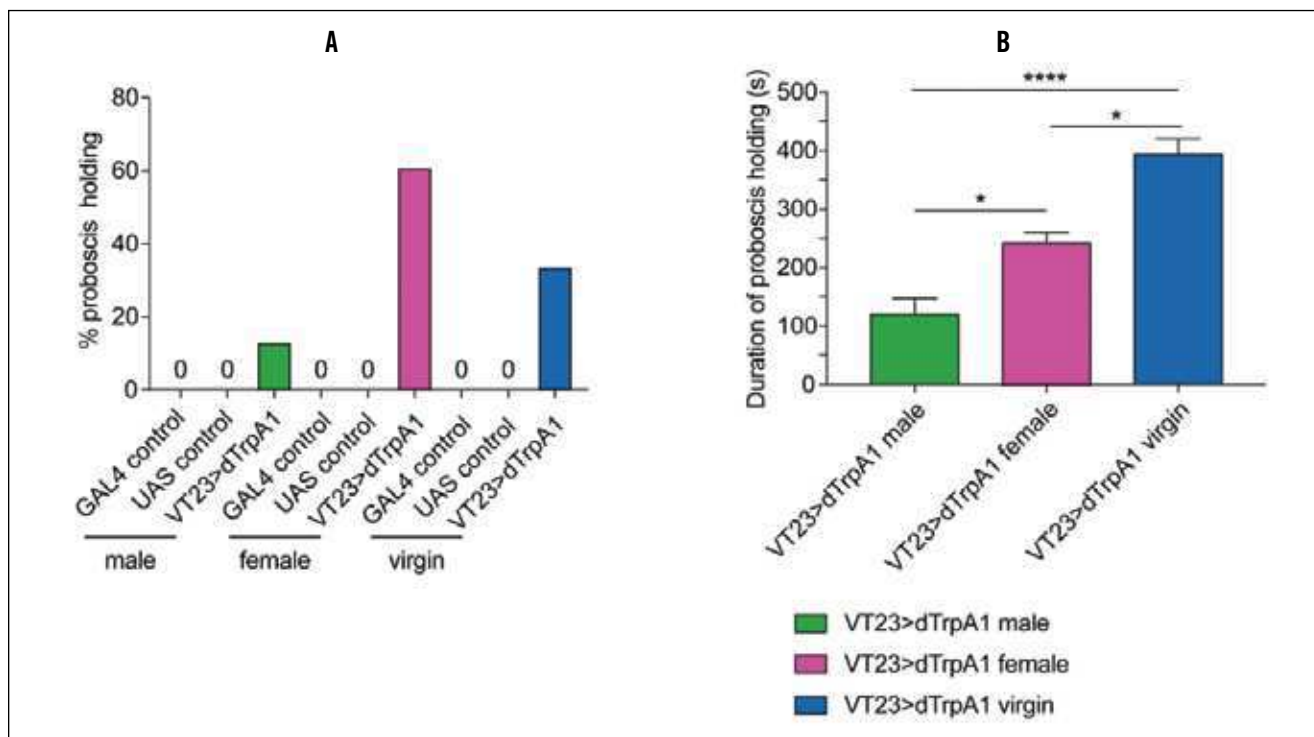


Figure 2. Activation of *VT23*-labeled neurons induces sexual dimorphic proboscis holding.
A. Flies were tested via the heat-activated proboscis extension reflex (PER) assay and their responses were recorded. An extension greater than one minute was counted as proboscis holding. *VT23>dTrpA1* flies showed varying degrees of participation among males (green), mated females (magenta), and virgin females (blue). The *GAL4* and *UAS* transgene controls showed no proboscis holding. N = 45-172.
B. Flies were tested via the heat-activated PER assay and the duration of their proboscis holding was timed. N = 10- 104. Differences between means of different groups were evaluated for statistical significance with the Kruskal-Wallis Test followed by the post hoc Dunn's multiple comparison test. * p < 0.05, **** p < 0.0001

DISCUSSION

In this study, we characterized the role of a subset of brain neurons labeled by *VT041723-GAL4* (*VT23-GAL4*) in feeding behavior in adult *Drosophila*. Thermogenetic activation of these brain neurons through expression of a heat-activated cation channel, dTrpA1, leads to the proboscis holding phenotype. Interestingly, the proboscis holding phenotype is sexually dimorphic. We observed that female *VT23>dTrpA1* flies exhibit proboscis holding more than males. In addition, the duration of proboscis holding is longer in both mated and virgin females than males.

The proboscis holding phenotype has not been described in literature. As reported by other research groups, when sugar-sensing neurons are thermogenetically activated, flies extend their proboscis many times as an indication of food acceptance (Du et al., 2016; Inagaki et al., 2014). In our study, we observed proboscis extension only one time without retraction when activating *VT23-GAL4* labeled brain neurons. Notably, many flies exhibited proboscis holding throughout the whole assay time (7 minutes). It is unclear whether proboscis holding indicates an attraction or aversion response. Given that there are multiple neurons labeled by this *VT23-GAL4* line, one way to interpret proboscis holding as an attractive response is that activation of some *VT23-GAL4* labeled brain neurons activate the appetitive taste circuit, leading to extension of the proboscis, while activation of other *VT23-GAL4* labeled brain neurons inhibit the motor program, preventing flies from retracting the proboscis. Alternatively, one could interpret proboscis holding as an aversive response, such as vomiting. Future research will need to be conducted to dissect these possibilities. For example, pre-feeding the flies before the heat-activated PER assay could potentially examine if food is vomited while proboscis holding.

Even though we do not have a conclusive answer for whether proboscis holding is an attraction or aversion response, we did reveal its sexually dimorphic nature. Female flies show proboscis holding significantly more than male flies. Why the sexual dimorphism exists will be another future research focus. Other research has found instances in which the neural circuitry of the fly brain differs between sexes (Kimura et al., 2005). Further experiments can compare the anatomy of *VT23-GAL4* labeled brain

neurons in male and female flies. If the number or pattern of *VT23-GAL4* labeled neurons is different between sexes, this could potentially account for the sexually dimorphic proboscis holding and provide an entry point for further investigation of which neurons are responsible for the behavior. In addition to mated females and males, we also tested whether the mating status would affect the probability of flies showing proboscis holding. Since there were much fewer virgin female trials, more trials for virgin females need to be done in the future to confirm if the results we obtained were significant. However, our initial results still suggest the sexually dimorphic nature of proboscis holding, since both mated females and virgin females showed more and longer duration of the behavior than males.

In this study, we establish the foundation for future analysis of simple sensory behavior through the genetic model organism *Drosophila melanogaster*, specifically in the taste circuit. We focused on one candidate transgenic line (*VT23-GAL4*) that might be involved in feeding behaviors. By utilizing the *GAL4/UAS* binary expression system and performing the heat-activated proboscis extension reflex (PER) assay, we uncovered a unique proboscis holding phenotype when activating these *VT23-GAL4* labeled brain neurons. We also demonstrated that proboscis holding is a sexually dimorphic behavior. The ample genetic resources and reagents in *Drosophila* will allow us to further narrow down *GAL4* expression to single neuron resolution, such as transcription repressor GAL80 or split-*GAL4* (Pfeiffer et al., 2010; Suster et al., 2004). This will allow us to pinpoint which individual neurons are involved in proboscis holding. Our results demonstrate a simple screening strategy that can be applied to other *VT-GAL4* lines to uncover different brain neurons processing taste information and mediate feeding behaviors.

ACKNOWLEDGEMENTS

This study would have not been possible without the mentorship of Drs. Anupama Dahanukar and Ryan Joseph and graduate student Yu-Chieh David Chen, to whom we are immensely grateful for their support and contributions over the past year and a half. Additionally, we would like to thank all the graduate and undergraduate students in the Dahanukar lab for their input.

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The Vagrancy of Race Suicide Through the Early Twentieth Century: Reimagining Fear

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ABSTRACT

The American Eugenics Archive defines race suicide as an alarmist term that describes, “when the birth rate within a so-called race dropped below the death rate...with the ultimate consequence that the “race” would die out.”¹ This article traces the ways in which fears and the concept of race suicide, a term coined by a sociologist committed to racial hierarchies, was reimagined by emerging black sociologist, W.E.B. DuBois who actively sought liberation from systematic racism in the Postbellum Era. This historical research seeks to analyze the ways in which fear among communities of color made claims of genocide inseparable from the histories of reproduction, birth control, sociology, race science, the Antebellum, and Jim Crow Era in the early twentieth-century. This is an attempt to provide a speculative history that allows fears of those most vulnerable within systematic oppression to be historicized, without the reigns of rigid, objectivity that act as a gatekeeper within the field of history. I argue that tracing fears of race suicide allows for a complicated and necessary reimagining of race science. The reimagining of race science allows us to see historical actors of color actively engaging in liberation struggle through what Britt Rusert calls oppositional science. Similarly, analysis of race suicide allows us to bridge what Judith Butler calls, the theory-practice divide.



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¹ “Race Suicide,” Eugenics Archive, April 29, 2014, <http://eugenicsarchive.ca/discover/connections/535eedb87095aa0000000250>.

INTRODUCTION

Historicizing an Emotion

The historicizing of emotions is a heavily debated subject within history as a discipline. Young historians are often taught to detach from their work. To remain as “objective” as possible is the quintessential goal. Only that which can be deemed “fact” is heavily emphasized. As their careers progress, however, young historians are exposed to the debates surrounding objectivity within the discipline. Despite this, many young (and professional) historians remain under the impression that detachment is the only ways to produce valid claims of knowledge. In this case, the promise of objectivity is as Donna Haraway asserts, a god-trick.² Moving past the false promises of complete objectivity allows for a new realm of historical analysis to emerge, the history of emotions, or rather, the history of ideas and concepts that point us toward specific experiences of emotion. This research will trace the understandings of twentieth-century fears surrounding the notion of race suicide.

In recent years many different psychological phenomena that can only be categorized as central to human emotions have been given room to be critically analyzed within history. Under this framework, emotions have come to be recognized as having specific, and identifiable histories. In this work, I attempt to trace fears surrounding race suicide. The American Eugenics Archive defines race suicide as an alarmist term that describes, “when the birth rate within a so-called race dropped below the death rate... with the ultimate consequence that the “race” would die out.”³ In understanding how fears of race suicide emerged and were reconfigured, the intertwining of multiple histories becomes clear and elucidates the ways in which oppositional science rooted in emotion created pathways to liberation from systematic oppression, specifically in the case of W.E.B. DuBois.

My research on ideas and fears surrounding race and reproductive autonomy began with blackgenocide.org. This particular website vehemently argued against the support of Planned Parenthood on the grounds that family planning

services sped up the process of genocide, a practice said to have been occurring in America in regards to African-Americans.⁴ The fear of genocide lies at the very core of this website. Nazi eugenics and the final solution are both mentioned in the site, the idea being that a similar plan is currently being pushed with regards to African-Americans, contraception and abortions provided by family planning services.⁵ As a result, the active support of family planning services changes genocide into active participation in race suicide. Fears of race suicide are not unique to one particular community, nor can the fear be generalized as encompassing the thought of a single community, this was merely my own [first] encounter with the phrase.

An emotion conceptualized—race suicide—allows for a complicated journey through fears in American history. It is important to acknowledge that many scholars have rejected the use of the term race suicide as an analytical concept, the reason being the term is rooted in racism, anti-feminism, connection to the eugenics movement, and its use of the category of “race” as a biological category. Despite this consensus by academics, race suicide has had and continues to have deep rooted psychological impacts on the relationship varying communities have with reproductive justice.⁶

Methodology

Academic discourse surrounding race suicide began in the 1970s. However, in the 70s, race suicide was only analyzed using race theory. Two works that best exemplify scholars’ oversimplification of race suicide are, Laura Umansky’s, *Motherhood Reconceived* and Robert G. Weisbord’s, “Birth Control and the Black American: A Matter of Genocide?” The aforementioned works both came to fruition in the 1970s and are of the select few that heavily engage with race suicide as it pertains to the 60s and 70s. However, due to these scholars’ use of a single framework of analysis, race suicide was oversimplified and ultimately, dismissed. In the contemporary period, there has been shift towards major emphasis on intersectional approaches to research, particularly in feminist studies. The ways in which I

² Donna Haraway, “Situated Knowledge: The Science Question in Feminism and the Privilege of Partial Perspective,” *Feminist Studies*, 3 (Autumn, 1988), 575-599. p. 584.

³ “Race Suicide,” Eugenics Archive.

⁴ “Blackgenocide,” accessed November 13, 2017, www.blackgenocide.org.

⁵ *Ibid.*

⁶ “Race Suicide,” Eugenics Archive.

analyze race suicide provides an intersectional analysis rooted in race and feminist theories as well as a framework centered in historiography. In providing an analysis that steps beyond race theory, I seek to change and reopen the dialogue surrounding race suicide.

In dismissing race suicide, scholars have consequently dismissed a massive topic, the fear of genocide present among communities of color. Indigenous, feminist scholar, Dian Million comments on Western historians tendency to dismiss claims of genocide, arguing that western notions of genocide remain solely with the experience of the Jewish community in the Holocaust.⁷ This ultimately causes most western historians to delegitimize other claims of genocide, both before and after the Holocaust. Genocide is solidified as something of the past, as a result, claims of genocide are not seen as worthy of historical analysis. I do not aim to prove race suicide is plausible, I do not aim to argue people of color can be complicit in race suicide by supporting contraception or family planning services. Rather, I seek to reopen the discussions of race suicide as a fear of genocide. A fear that has been present in many communities as a result of settler colonialism. This fear, this dismissed topic, has opened the door to different paths of liberation which makes it worthy of critical analysis. As a result, outright dismissal is irresponsible and a continued silencing on behalf of historians and other scholars.

Based heavily on archival research, this article will engage heavily with the concept of race suicide. I use the University of Massachusetts digital archive of W.E.B. DuBois' published papers, letters and writings alongside the works of controversial sociologist Edward A. Ross in order to understand race suicide in its conception and elucidate the ways in which it was reimagined in spaces of color that allowed it to change the face of eugenics in a way that opened the path to liberation from systematic racism. Embedded within DuBois' digital archive is also the work of the Birth Control Federation of America, which will also factor heavily in explaining DuBois' role in this work. This historical research article will also draw on secondary monographs of prominent historians such as: Aldon Morris and Britt Rusert in order to provide critical context and

the backdrop needed to understand the different voices engaging with race suicide at the turn of the century. Finally, I draw inspiration from Dian Million's indigenous, feminist concept of "felt theory." Felt theory will give the tracing of race suicide a framework that will allow me to trace race suicide through the fears of those who felt it. Historical reigns of objectivity will be loosened and I will allow fears of communities of colors, as well as their anger and frustration surrounding inner community conflict to be heard. I will not delegitimize fears of genocide, rather I will seek their origins in order to understand why a term coined in the twentieth century continues to be pervasive in the modern day. In seeking the roots of fear, I will elucidate how different historical actors have reacted to and addressed race suicide and how these reactions shaped the fight against racist, masculinist thought, as well as the fight for reproductive autonomy.

I propose a speculative history, one that does not offer definitive answers as to why race suicide became such a prevalent topic for W.E.B. DuBois in the twentieth century, though I will attempt to tackle this question as best I can. I offer a history that seeks to acknowledge fears of genocide within a settler state built on systematic racism. I offer a history that seeks to understand how these fears were regarded, used and reimagined in order to provide a pathway towards liberation. To begin this long journey, I will turn my attention to W.E.B. DuBois and his sociological interaction with notions of race suicide.

From Fear to Fallacy:

At the end of the nineteenth century, the field of sociology began emerging in America, its emergence began less than a century after emancipation.⁸ The emancipation of former slaves did not address the status of black Americans in society, and at the turn of the century, circulating rhetoric seemed to continue the subjugation and systematic oppression that had encompassed African-American life in America. Slavery had shaped the ideas of the white populace and popular scholarly discourse further pushed a notion of black inferiority, this push was especially present in the emerging world of sociology.⁹ Historian Aldon Morris comments on the role of sociology in its emergence,

⁷ Dian Million, "Felt Theory: An Indigenous Approach to Affect and History." *Wicazo Sa Review*, Volume 24, Number 2, Fall 2009, pp. 69

⁸ Aldon Morris, *The Scholar Denied: W.E.B. DuBois and the Birth of Modern Sociology* (California: University of California Press, 2015), 6.

⁹ *Ibid.*, 7.

stating that the field struggled to get a foothold within academia and was tasked with providing explanations for race relations along with addressing challenges of immigration and reproduction.¹⁰ Thus, it is not surprising that it was within this climate that race suicide was first coined in an America shaped by years of one of the most profitable slave regimes ever enacted.

In 1901, sociologist Edward A. Ross followed the lead of many white sociologists and published perhaps his most well known work, *The Causes of Race Superiority*, in this work Ross coined the term “race suicide.”¹¹ Ross’ work centered on notions of racial hierarchies, and it was within these hierarchies of “superior” and “inferior” races, that competition of race survival emerges.¹² Race suicide occurred when “superior” races, in this case white Americans, no longer felt the need to reproduce due to a standard of comfort, which could lead to the extinction of the race.¹³ In the early 1900s, America was a country coming to terms with mass social and economic change. Ross’ work directly reflects what the field of sociology encompassed at this time, white hegemonic ideas of racial superiority and the fervent need to protect white hegemony from immigrants and “inferior” races, in this case the recently emancipated black population. Fear is at the center of Ross’ work. Fear is meant to push towards pronatalist attitudes which were meant to ease the minds of anxious white Americans whose lives and views had been shaped by the slave era. Ross’ notion of race suicide was an attempt to grapple with rapid changes in American society that ultimately followed the guidelines of the new emerging field of sociology, which at the time sought to perpetuate ideas of racial hierarchies.

From 1892 to 1894, DuBois, like many scholars interested

in the social sciences at the time, traveled to Berlin to train under German masters of the social sciences.¹⁴ It was during this training that DuBois was taught to reject studies that validated themselves through the claims of “natural laws.”¹⁵ At the time of DuBois’ studies in Europe, the eugenics movement was rising at the same time that Jim Crow America was beginning to unfurl.¹⁶ The two systems helped reinforce the other, eugenic ideas were reflective of the new ideals of Jim Crow America, as a result, eugenic thoughts seeped into the new field of sociology.¹⁷ The nature of American sociology now stood at odds with the ways in which DuBois had been trained in Germany. DuBois had been trained to resist research that sought to prove itself through things like “natural” laws, something the sociology movement in America was notorious for doing, especially after internalizing eugenic ideologies.¹⁸

DuBois engaged heavily with disproving eugenic ideals at the turn of the century, but he also involved himself greatly with the emerging birth control movement. At a glance, DuBois’ connection to the birth control movement may seem separate from his ties to sociology and eugenics, but the birth control movement was the way in which DuBois actively challenged the ideas of Ross that had seeped into the veins of Americans across racial lines. Sociology began merging with eugenics in this period. As a result, eugenicists’ obsession with reproduction rates made the link between contraception, sociology and eugenics inseparable. As a result, in June of 1932, DuBois gave the Birth Control Federation of America (BCFA) permission to publish a previous article he had provided them in their eighth edition of their journal, entitled, “The Negro Number.”¹⁹ The Negro Number was part of the Birth Control movement’s effort to introduce contraception to black southerners.²⁰ The eighth edition of

¹⁰ Ibid.

¹¹ Edward A. Ross,” American Sociological Association, accessed November 20, 2017, <http://www.asanet.org/about-asa/asa-story/asa-history/past-asa-officers/past-asa-presidents/edward-ross>, paragraph 1.

¹² Edward A. Ross, “The Cause of Race Superiority,” *The Annals of the American Academy of Political and Social Science*, 18 (Jul., 1901),: 67-89.

¹³ Ibid, 88.

¹⁴ Aldon Morris, *The Scholar Denied: W.E.B. DuBois and the Birth of Modern Sociology*, 17.

¹⁵ Ibid, 21.

¹⁶ Ibid, 19.

¹⁷ Many sociological journals reprinted the work of Francis Galton, an English scientist who proclaimed he had developed the science of eugenics.

¹⁸ Ibid, 22.

¹⁹ W.E.B Du Bois, Charles S. Johnson, George S. Schulyer, “Birth Control Review Vol. Xxii, No. 6, June 1932,” accessed January 24, 2018,

<https://lifedynamics.com/app/uploads/2015/09/1932-06-June.pdf>

²⁰ Ibid, 163.

the journal included prominent African-American leaders' positive thoughts on the possible impact of contraception. It is in this article, which had been written prior to 1932, that DuBois sought to disprove fears surrounding race extinction through race suicide. The journal specifically mentions the "Negro Problem" which not only referred to the disenfranchisement of black communities, but also the bigger notion of incorporating African-Americans into American society, the notion that had loomed over the twentieth-century since emancipation in the nineteenth-century.²¹ This journal and broader movement's addressing of the "Negro Problem" was perfectly in step with what DuBois had been attempting with sociology since the early 1900s. It also allowed DuBois the perfect arena to challenge eugenic ideologies, the birth control movement was, by default, at odds with eugenicists of the time who condemned the use of contraception. By allowing the journal to publish his article, DuBois would be directly in relationships with groups actively opposing ideas pushed by eugenics, thus it makes perfect sense that this is where DuBois actively sought to disprove race suicide.

In his article, "Black Folk and Birth Control," DuBois addresses the high reproductive rates among African-Americans, arguing that this high birth rate is rooted in false fears of race extinction.²² DuBois refers to fears of race extinction as the fallacy of numbers. DuBois believed African-Americans were buying into a fear of race extinction which would only be furthered by low reproductive rates. On the subject, DuBois comments, "they [African-Americans] are quite led away by the fallacy of numbers. They want the black race to survive. They are cheered by a census return of increasing numbers and a high rate of increase. They must learn that among human races and groups, as among vegetables, quality and not mere quantity that counts."²³ This fear among black communities presented by DuBois parallels the fears among whites presented by Ross. In both instances, there is a fear of low birth rates leading to race extinction. Ross directly states that not actively reproducing is race suicide, DuBois did not, nor did he have to, this already seemed to be a solidified belief among disenfranchised

communities of African-Americans seeking to survive through reproduction. In DuBois' writing, however, there is not a fear of displacement or an acknowledgement of racial hierarchies. As mentioned before, DuBois actively challenged notions of racial hierarchies, so this would not have been present in his discussion of race survival. For African-Americans, fears of survival were understandably rooted in familial structures of the Antebellum era. DuBois specifically commented on the roots of genocidal fears, stating, "Tradition of early marriage and large families has put grave strain on a budget...not merely to maintain, but to improve...standard of living. As slaves, every incentive was furnished to raise the largest number of children possible."²⁴ The slave era was wrought with violence and terror, family structures were constantly restructured, ripped apart and restructured again. The production of more slaves was the production of commodities, a production actively encouraged by slave masters to reap the most economic benefits out of the bodies they owned. Similarly, slaves actively resisted through the nurturing and maintaining of what familial structures they could achieve. DuBois acknowledges this and understands that constant turmoil and violence within familial structures of the slave era initially introduced the fear of race extinction into African-American communities which in turn, emphasized the family unit as means of resistance and survival. It is significant to note DuBois' emphasis on the Antebellum era, it is an instance in which DuBois actively sought to contextualize social issues that were presenting themselves at the time. The acknowledgment of the fears of genocide and race extinction do not emerge from a hierarchical competition among races, for DuBois, fears emerge out of the lasting effects of a slave regime which terrorized, threatened and murdered countless black bodies. Acknowledging the lasting effects of the slave era disproves Ross' belief in hierarchies and centers fear of extinction on legitimate claims that reference past atrocities against African-Americans. In contextualizing, DuBois delegitimizes Ross, while assuring African-Americans that while he understood their fears, contraception was still key to liberation.

²¹ Ibid.

²² Ibid.

²³ Ibid.

²⁴ Ibid.

Similarly, the mere act of conveying this message through the Birth Control Review, ensured that DuBois would not only reach the black community, but that he would also reach any readers of the journal which very well could have included sociologists as well as eugenicists seeking to oppose the material.

The factors that lead to fears of race suicide and extinction are different in white and black spaces, highlighting the fluidity of the idea, but the solutions and fears mirror one another across different historical settings. Ross argued that race suicide was a legitimate fear that could be solved through pronatalism. Similarly, African-Americans also saw their survival wrapped up in high birth rates. DuBois, however, sought to altogether disprove fears of race suicide and extinction in his discussion of high reproductive rates among African-Americans. Addressing fears of race survival as fallacy signals DuBois' silent, but strong dismissal of a fear so vehemently pushed in white, sociologist circles. The solutions presented to combat race suicide and extinction presented by Ross are also actively challenged by DuBois.

Unlike Ross, DuBois did not believe high rates of reproduction, would ensure race survival. DuBois vehemently argued against the use of high reproductive rates to combat the new form of violent and racist society. DuBois allowed the publication of his article in the edition of the Birth Control Review actively seeking to remedy high death rates of black mothers as well as outbreaks of disease in rural black communities through contraception.²⁵ DuBois comments, "The mass of ignorant Negroes still breed carelessly and disastrously, so that the increase among Negroes, even more than the increase among whites, is part of the population least intelligent and fit, and least able to refer their children properly."²⁶ DuBois' statement, though elitist in nature, continues to be wrapped up in his idea of race elevation. For DuBois, contraception was an active way to allow African-American women time to physically heal after childbirth while simultaneously allowing families ample opportunity to raise children in a manner that would ultimately promote healthier ways of navigating

life in an oppressive state. Thus, the fear of extinction was unsubstantiated. Race suicide was not possible because fears of race extinction were mere fallacy and in opposition to liberation.

Conclusion: Counter Sciences and Reimagining the History of Science.

At the dawn of the century, W.E.B. DuBois was determined to reimagine the social sciences in a way that would open pathways of liberation to African-Americans. DuBois' training in sociology allowed him to return to America ready to challenge Jim Crow America. In allowing his works to be circulated by the birth control movement, DuBois ensured his message reached those he cared most for and those he was determined to undermine. DuBois' opposition came at a time in American history where African-Americans were mitigating the terms of their identity. Emancipation was said and done, but this did not guarantee incorporation into a society shaped by oppression, subjugation and white hegemony. African-Americans were grappling with this at the turn of the century and thus, fears that understandably stemmed from the slave era translated into Postbellum America. Fears of race extinction and stagnation that resulted in active participation in what Ross deemed race suicide emerged among African-Americans, but it was not without its challengers. DuBois exemplified what Britt Rusert referred to as oppositional sciences.²⁷ These oppositional sciences are characterized as seeking to make an intervention in scientific discourse, DuBois was an active participant in these oppositional forms of science, particularly, speculative science which sought to mediate on the contingencies of black subjectivity and existence.²⁸

Seldom is opposition at the forefront of discourse surrounding the history of race science. Perhaps like claims of genocide, ideas of race science are also wrapped up in a particular time frame of world history. As a result, it is imperative to trace the histories of fear surrounding race suicide. Race suicide allows for a new narrative in the histories of race science to emerge, oppositional narratives. Black participation in racial science allowed for the reclaiming and revising of beliefs meant to oppress.

²⁵ Margaret Sanger, "Planned Parenthood Leaflet, Ca. 1941," accessed December 15, 2017, <http://credo.library.umass.edu/view/full/mums312-b094-i139>.

²⁶ W.E.B Du Bois, Charles S. Johnson, George S. Schulyer, "Birth Control Review Vol. Xxii, No. 6, June 1932."

²⁷ Britt Rusert, *Fugitive Science: Empiricism, and Freedom in Early African American Culture* (New York: New York University Press, 2017), pg. 18

²⁸ *Ibid.*

Oppositional counter sciences did not disappear after the 1930s and neither did fears of race suicide, DuBois' challenging of racial sciences in the 1930s would be done again in the 1970s by black feminists seeking to oppose race suicide within their activist circles and within the mainstream feminist movement.

To address black feminism and race suicide is the task of another long and exciting project. Further research is needed on the subject and I intend to devote myself to the aforementioned relationship in further studies. However, I urge readers and scholars to now turn their attention to all that could be lost if race suicide is dismissed as a concept of analysis. Race suicide tells a story of fear. In tracing race suicide, we can see how claims of genocide became attached to fears rooted in violence and survival. Ultimately, race suicide intertwined multiple histories and made them emotional, which ultimately led to erasure. Perhaps race suicide was too tied to emotion to be relevant for the rigid notions of detachment that often serves as a gatekeeper to history. If history is to move forward as a discipline, it must learn to come to terms with its relationship to

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emotions. History is no less valid when centering on lived experiences, and emotions. Lived experience is after all, the goal of liberatory feminism and what should be the goal of a liberated field of history.

The silencing of race suicide has silenced invaluable instances of opposition and mass changes in liberation struggles. Understanding the contentious past of race suicide allows us to understand its re-emergence in the contemporary moment. Bodies are still under subjugation in the twenty-first century and elucidating the contentious relationship of America as a settler state with the history of reproduction allows for new pathways towards liberation to be made visible as they were in the 30s, and again in the 70s. Race suicide shows the work still needed within both academia and activism, within theory and practice. Feminist scholar Judith Butler proposed the idea of theory-practice divide, in which work in the realm of academia and activism remain separate. The analysis of race suicide allows for one instance of bridging this divide in a way that seeks a feminist abolition of institutional violence and oppression.

Dana Simmons, "Impostor Syndrome, a Reparative History," *Engaging Science, Technology, and Society* 2 (2016), 106-127.

Dian Million, "Felt Theory: An Indigenous Approach to Affect and History." *Wicazo Sa Review*, Volume 24, Number 2, Fall 2009.

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W.E.B Du Bois, Charles S. Johnson, George S. Schulyer, "Birth Control Review Vol. Xxii, No. 6, June 1932," accessed January 24, 2018, <https://lifedynamics.com/app/uploads/2015/09/1932-06-June.pdf>

ENDNOTES

1. Donna Haraway, "Situated Knowledge: The Science Question in Feminism and the Privilege of Partial Perspective," *Feminist Studies*, 3 (Autumn, 1988), 575-599, p. 584.
2. The examples listed here specifically reference, Dr. Dana Simmons' article entitled "Impostor Syndrome, A Reparative History." As well as Jennifer Cole and Lynn M. Thomas' book entitled, "Love in Africa," which traces the history of love, sex and intimacy in Africa.

3. Dana Simmons, "Impostor Syndrome, a Reparative History," *Engaging Science, Technology, and Society* 2 (2016), 106-127.
4. "Blackgenocide," accessed November 13, 2017, www.blackgenocide.org.
5. *Ibid.*
6. "Race Suicide," *Eugenics Archive*, April 29, 2014, <http://eugenicsarchive.ca/discover/connections/535edb87095aa0000000250>.
7. Dian Million, "Felt Theory: An Indigenous Approach to Affect and History," *Wicazo Sa Review*, Volume 24, Number 2, Fall 2009, pp. 69
8. Aldon Morris, *The Scholar Denied: W.E.B. DuBois and the Birth of Modern Sociology* (California: University of California Press, 2015), 6.
9. *Ibid.*, 7.
10. *Ibid.*
11. Edward A. Ross," American Sociological Association, accessed November 20, 2017, <http://www.asanet.org/about-asa/asa-story/asa-history/past-asa-officers/past-asa-presidents/edward-ross>, paragraph 1.
12. Edward A. Ross, "The Cause of Race Superiority," *The Annals of the American Academy of Political and Social Science*, 18 (Jul., 1901),: 67-89.
13. *Ibid.*, 88.
14. Aldon Morris, *The Scholar Denied: W.E.B. DuBois and the Birth of Modern Sociology*, 17.
15. *Ibid.*, 21.
16. *Ibid.*, 19.
17. Many sociological journals reprinted the work of Francis Galton, an English scientist who proclaimed he had developed the science of eugenics
18. *Ibid.*, 22.
19. W.E.B Du Bois, Charles S. Johnson, George S. Schulyer, "Birth Control Review Vol. Xxii, No. 6, June 1932," accessed January 24, 2018, <https://lifedynamics.com/app/uploads/2015/09/1932-06-June.pdf>
20. *Ibid.*, 63.
21. *Ibid.*
22. *Ibid.*
23. *Ibid.*
24. *Ibid.*
25. Margaret Sanger, "Planned Parenthood Leaflet, Ca. 1941," accessed December 15, 2017, <http://credo.library.umass.edu/view/full/mums312-b094-i139>.
26. W.E.B Du Bois, Charles S. Johnson, George S. Schulyer, "Birth Control Review Vol. Xxii, No. 6, June 1932."
27. Britt Rusert, *Fugitive Science: Empiricism, and Freedom in Early African American Culture* (New York: New York University Press, 2017), pg. 18.
28. *Ibid.*

Psychoanalytic Feminism and the Depiction of Women in Surrealist Photography

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ABSTRACT

Surrealism, an art movement of the early twentieth century, was heavily influenced by psychoanalysis. The psychoanalytic theories that influenced Surrealism were based primarily on the research of Sigmund Freud. Freud's research began with case studies on patients with hysteria, a predominantly female diagnosed mental disorder. From his clinical observations of hysteria, Freud developed his theories on unconscious drives and psychosexual development. André Breton, the leader of the Surrealist movement, first became acquainted with Freud's ideas during the First World War. After his return to France from the war, Breton's interest in avant-garde art and distaste for Europe's high culture led him to start the Surrealist movement. Breton declared psychoanalysis the basis of Surrealism in the First Manifesto of Surrealism, believing that Freud's ideas had the potential to revolutionize culture. For the Surrealists, adopting psychoanalysis as a doctrine of change resulted in a reinforcement of sexist stereotypes and discrimination against women that was rooted in Freud's theories. While the Surrealist movement became notorious for being male dominated and misogynistic, their idealization of Freud provided justification for their prejudiced beliefs. In this paper, Salvador Dalí's photo collage, *The Phenomenon of Ecstasy*, is analyzed to exemplify the translation of psychoanalytic ideas into sexualized and fantasy-like depictions of women in Surrealist artwork. The conducted research provides insight to the repercussions that Freud and psychoanalysis had on women in the Surrealist art community.

Keywords: Surrealism; André Breton; Photography; Avant-garde art; Salvador Dalí; Feminism; Psychoanalysis; Sigmund Freud



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INTRODUCTION

The photographic artwork of the early twentieth century Surrealist movement was defined by illusionistic images evoked from the artist's unconscious mind. Through the camera, Surrealist artists captured peculiar scenes of people and objects in faux dream realms. This way of using the camera was paradoxical, as the device's intended use to document reality was replaced with the artists' manipulation of the photograph in conjunction with psychoanalytic theory to translate their unconscious dreams into an altered reality. A visual analysis of Salvador Dalí's photo collage, *The Phenomenon of Ecstasy*, reveals the ways in which psychoanalytic theory was applied by Surrealist artists to create bizarre photographic images, often exploiting female subjects at the center of the works. From a contemporary feminist perspective, the photographed women in Surrealist works were purposely sexualized and used as passive muses of the artists' fantasies. Arguably, the misogynistic elements at the foundation of psychoanalysis had a substantial influence on Surrealist images. Freud's theories functioned as an inspiration and justification for Surrealist artists, like Salvador Dalí, to derogatorily portray women in photographic artworks.

Freud and Psychoanalysis

The version of psychoanalytic theory that influenced Surrealism was based primarily on the research of Sigmund Freud, a leading researcher in psychoanalysis. In the late nineteenth century, Freud began his psychological research through an apprenticeship under the French neurologist Jean-Marc Charcot in Paris, France.¹ Charcot was performing research on patients to understand and treat hysteria, a mental illness believed to derive from a weak neurological system and the repression of traumatic events.² Patients diagnosed with hysteria exhibited symptoms of excessive anxiety, exaggerated emotional responses, and disturbed somatic functioning. Historically, the illness was diagnosed predominantly in female patients, as the exhibited symptoms were dramatic in appearance and, for the early twentieth century, culturally seen as a manifestation of femininity. To treat his patients, Charcot used hypnosis in an attempt to bring suppressed memories from the unconscious to the conscious mind.

Freud began his own research on hysteria primarily through

clinical observations of Bertha Pappenheim, a female patient of Josef Breuer, a physician and peer of Freud. Pappenheim was given the pseudonym Anna O. by Freud and Breuer for their published book, *Studies on Hysteria* (1895).³ Clinical observations of Pappenheim, and patients with similar symptoms, led Freud and Breuer to originally hypothesize that hysteria was caused from unresolved emotions and experienced trauma. Later on in his research, Freud revised the theory to include psychic conflict related to sexuality as a primary cause.⁴ From the research on hysteria, Freud developed his original psychoanalytic theory of the mind. Freud's psychoanalysis theorizes that unconscious thoughts and motivations, rooted in primitive drives toward sex and aggression, are the underlying cause of human behavior.⁵ This definition of psychoanalysis became Freud's preliminary explanation of the psyche's mechanisms. Dream interpretation, free association, and hypnosis were some of Freud's treatment approaches used to reveal the mind's unconscious conflict. These Freudian techniques were manipulated in Surrealist art practice to exemplify the irrationality of human behavior and bring unconscious desires to visual awareness.⁶ While these psychoanalytic ideas inspired the Surrealists, the theories on hysteria and animalistic impulses, rooted in cultural misogyny, had negative repercussions on the movement's art. The repercussions translated into the movement's shocking depictions of women as sexually objectified and submissive to the viewer.

Freud's interests soon progressed toward psychosexual development, a concept that has caused considerable controversy since its first appearance in Freud's *Three Essays on the Theory of Sexuality* (1905). Psychosexual development theorizes that humans have an instinctual libido that develops during childhood.⁷ According to the theory, humans progress through five phases of sexual development, beginning at birth and continuing after puberty. The controversial aspect of the theory, other than its reference to sexual desire in children, comes from the third phase of psychosexual development: the phallic phase. Freud states that during the phallic phase, between the ages of three and six, there is a divergence between male and female psychosexual growth.⁸ This divergence occurs when children become conscious of the biological difference between male and female genitalia, causing

the emergence of the Oedipus complex. According to the theory, females develop penis envy and resentment toward the mother for not providing her with a penis.⁹ On the contrary, males compete with the father to sexually possess the mother and subsequently develop castration anxiety, or fear of emasculation by the father.¹⁰ The fundamental misogyny in these Freudian ideas on sexuality translated into the portrayal of women in Surrealist photography as sexual objects for the viewer. The Oedipus complex acted as a justification to depict women as passive and powerless in the images, inferior to the male artist.

Surrealism Inspired by Psychoanalytic Theory

Surrealists were fascinated with Freudian ideas and established them as the basis of their artistic methods with the guidance of André Breton, the founder of the movement. While stationed as a psychiatric aide in the First World War, Breton first became interested in Freud's theories through a fellow psychiatrist interested in dream interpretation.¹¹ Through individual study, he became acquainted with Freud's observational methods and applied them in practice during interactions with his war-time patients.¹² Dream interpretation and free association became his primary interests of study and would become a fundamental part of the Surrealist method. After his departure from the war, Breton's curiosity about abstract thought and his frustrations with France's bourgeois culture led him to join the Dada movement. Dadaism, a precursor and inspiration to Surrealism, began as an avant-garde art movement in reaction to the First World War. The movement protested bourgeois society, acting against logic, reason, and aestheticism, the elements that maintained the façade of high culture.¹³ Breton combined Dadaist ideas and psychoanalytic theory to develop the foundation of Surrealism.

Breton officially launched the Surrealist movement in 1924, establishing psychoanalysis as the basis of the movement. Breton wrote the *First Manifesto of Surrealism* (1924) to declare the purpose of Surrealism as a revolutionary movement against structured culture. The manifesto defines Surrealism as "psychic automatism in its pure state," with automatism as a reference to Freud's idea of using free association to reveal the unconscious mind.¹⁴ In this founding text, Breton discusses Freud's theories on

dream interpretation as fundamental for understanding the deeper meaning of existence, as "the human explorer will be able to carry his investigation much further, authorized as he will henceforth be not to confine himself solely to the most summary realities."¹⁵ Breton's text denounces the rational standards of the bourgeois society and proposes the untamed methods of Surrealism as revolutionary modes of understanding human existence. The manifesto's denouncement of cultural standards meant acting on the inherent behaviors of the mind's unconscious desires, rather than conform to the conventional norms of social conduct. As early twentieth century European culture was progressing toward the liberation of women, the manifesto's condemning of social norms meant not allowing women to be independent of men. In Freudian terms, women were deemed inherently incompetent on their own and meant to be the physical possession of man. The manifesto's propaganda served as a guide for Surrealist practices, claiming psychoanalysis and the rejection of social structure as essential in the movement's model.

Sexism and Freud's Psychoanalysis

While Freud's theories contributed to the understanding of the human mind, his ideas had consequences that perpetuated sexism against women. In Freud and Breuer's hysteria case study on Bertha Pappenheim, from *Studies on Hysteria*, Pappenheim exhibited symptoms characteristic of the overly emotional and highly sensitive female stereotype. The disorder was studied and treated by culturally biased men whose medical status allowed for the gendered stereotype to become scientifically validated and pathologized as hysteria.¹⁶ Furthermore, the root of hysteria's etiology was thought to center in female patients' genitalia as a result of sexual repression, a diagnosis appropriate for a culture that deemed women sexually passive.¹⁷ This representation of women by Freud, an authoritative figure in the scientific community, validated the social construct of female inferiority and influenced Surrealist's perspective of women.

Freud's formulation of the unconscious mind's drives toward sex and aggression maintained the derogatory beliefs originating from hysteria case studies. Freud suggested that human desires toward sex and aggression are the main components of all human behavior, based on

an animalistic instinct to act on these tendencies.¹⁸ The theory is simultaneously a symptom and an affirmation of patriarchal society in the early twentieth century. Women were a socially vulnerable population in European society, and given his influence, Freud's ideas on sex and aggression were highly influential to the treatment and representation of women. The consequences of his ideas would be especially apparent among the Surrealist community, where misogyny was practiced and accepted. The movement was notoriously difficult for female artists to join, as the male artists that managed the movement wanted to maintain their masculine idealizations of femininity.¹⁹

Furthermore, theories on psychosexual development portray women as sexually passive and envious of their male counterparts, ideas solely hypothesized by male scientists.²⁰ Based on the evolutionary aspect of the theory, a woman's primary role is to bear the man's offspring. Her body is a vessel used to produce children in order to continue the male's bloodline. Freud claims that through the Oedipus complex the unconscious mind formulates these assigned gendered roles for sexuality, believing that they are not devised by culture, but are biologically grounded.²¹ The Oedipus complex illustrates the translation of the era's sexist ideals into scientific theories of gendered sexuality. Men have the penis, the active form of sexuality, while women have the vagina which acts passively to sheath the penis in the act of conception. The man experiences fear of castration because he does not want to become the passive woman, while the woman envies the man's penis, for she is in the role of passive female, an object only used to suffice the man's sexual and child-bearing needs. This theorized scenario strips the woman of her autonomous sexual identity. There is only the man's sexual identity and she is a secondary part to that role, as was exemplified in the Surrealist's use of the female muse.

Misogyny Rooted in the Surrealist Movement

While sexism in the Surrealist movement did not originate from Freud's theories, his credibility as a scientist strengthened and justified the misogynistic beliefs that were already in place within artistic communities. These beliefs were rooted in the sexist constructs of the era's culture, trickling over to Surrealism from previous art movements, Futurism and Dadaism, where women struggled to be seen

as credible artists. While the first wave of feminism was rising in France during the 1920s, the Surrealists, insisting on their opposition to socio-cultural conventions, saw the movement primarily as bourgeois nonsense.²² The Surrealists sought to maintain the oppression of women and continued to exploit their female subjects for the sake of creativity and the psyche's unconscious fantasies. This resulted in the hindrance of female avant-garde artists from participating in Surrealist exhibitions and publishing in Surrealist journals.²³ The principal role for a woman within the movement was as the creative stimulus for a male artist.

The female subjects in Surrealist art were dehumanized, portrayed as the muse, the seductress, and *la femme-enfant*. For example, in Breton's novel *Nadja* (1928), *Nadja* becomes the inspiration for the creative work, or the muse of the artist. In the novel, Breton writes of his love affair with *Nadja* and her demise into psychosis. *Nadja*, like the women in Surrealist photographs, becomes the focal point of the work, but her identity is never truly revealed. She is manipulated and reassigned an identity that fits with the creative narrative of Breton, the male artist. If Breton was fascinated by *Nadja*'s mental disorder, it was because he could appropriate her story for his own creative use.

Breton's use of *Nadja* for his own creative purposes was not uncommon in a movement dominated by heterosexual men seeking to transpose their sexual fantasies into reality. Often, the women in Surrealist photographs are the epitome of *la femme-enfant*: young, naïve, and pure, with child-like mystique that the Surrealists believed had the ability to provoke the unconscious and irrational thoughts of the artist.²⁴ In other cases, she becomes a seductress, sexually tempting men to view her as an object of their desires. The fantasy depictions of female subjects as nude, vulnerable, and passive all contribute to the male artist's dreams of idealized femininity. Their surreality lies not only in the irrationality of the photographs, but also in the viewer's ability to act as a voyeur, peering into a captured moment of eroticism.

Psychoanalytic Feminist Analysis of Dalí's *The Phenomenon of Ecstasy*

Misogynistic principles were rooted in the creation and maintenance of both psychoanalysis and Surrealism, with

the Surrealist movement's portrayals of women heavily influenced by Freud's sexist theories. For the purpose of this essay, *The Phenomenon of Ecstasy* (fig. 1), a photo collage by Salvador Dalí, will be analyzed to understand the application of psychoanalytic concepts in relation to Surrealist artists' portrayal of women in photography.

The Surrealists were fascinated by mental illness, exemplified by Breton's presentation of Nadja, as they believed the mentally ill to have special insight into the unconscious. Similarly, in *The Phenomenon of Ecstasy*, Dalí romanticized the *attitudes passionelles* of women clinically diagnosed with hysteria. The phrase, *attitudes passionelles*, was used by Surrealists to describe the experience of ecstasy that supposedly resulted from hysteria.²⁵ The artists saw the disorder as an ultimate form of expression, rather than a pathological occurrence. A dedication to the fiftieth anniversary of hysteria was celebrated in a two-page spread of the eleventh edition



Figure 1. Salvador Dalí, *The Phenomenon of Ecstasy*, 1933.

of *La Révolution surréaliste* (March 1928), a Surrealist publication, where the article's first line refers to the disorder as "the greatest poetic discovery of the nineteenth century."²⁶ Breton, the director of the journal, chose to include six photographs, spanning a page and a half of the article, from a female patient's case study on hysteria. Each image is of the same woman, confined to her bed, displaying different theatrical expressions and gestures that appear illogical in sequence. Superficially, the Surrealists' views on hysteria might appear reverent. But beneath the surface lies the discrimination of a marginalized population of suffering women, and the appropriation of their experiences in furtherance of the movement's agenda.

Dalí's *The Phenomenon of Ecstasy* has implications parallel to the article on hysteria published in *La Révolution surréaliste*.¹¹ The collage uses images of over twenty women in states of euphoria. The images are cropped to only show the women's faces, placing complete emphasis on the figures' expressions. Many of the female subjects in the work are photographed gazing upward with their lips slightly parted, a typical hysteria pose as documented by Charcot and Freud from their case studies.²⁷ At first glance, the work appears chaotic from the collaged photos' varying sizes and colored tints, a device that mirrors the disarray of hysteria. Despite the disorganization, the collage's content is shocking and appeals to the curiosities of the viewer. Additionally, Dalí has placed photographs of a floating chair and a decorative staff. These images of inanimate objects are irrational in the work's schema, breaking the serial iconographic structure of the collage and adding to the chaotic tone.

The viewer's gaze is first drawn by the largest image at the center of the collage, the focal point that guides the viewer through the work. The woman in the center image appears peaceful and angelic, the typical *femme-enfant*. Below her image, Dalí has placed a photo of a marble statue bearing a similar blissful expression. The placement of these two images has allowed the viewer to make the comparison of the ecstatic women to that of a statue. She appears pure, virginal, and innocent, inviting the viewer to gaze at her sexualized expression. Repeatedly, the depiction of the youthful and exuberant woman appears in multiple photos around the focal image. Each of the photos of the

women are angled to make the subject appear as though she is laying down. The women become the epitome of the passive female, controlled by their emotional state and need for sexual satisfaction. In Freudian terms, these women are the objects of desire, used primarily to fulfil the sexual fantasies of the observer.

The Freudian theme of active male and passive female emanates throughout the work. In the bottom left corner an image depicts a man examining two delirious females. It seems that the image comes from a pornographic photo that Dalí has cropped and included in the collage, placing emphasis on the figures heads. The cropping of the image takes the subjects out of their original context and assigns them a new identity. The man appears to take on the role of clinician while the women are his patients. The image draws attention to the proximity of sex and hysteria, as the photo is cropped directly from a pornographic scene of aroused women, yet appears to depict an objective clinician examining two patients. The man's posture and gaze give him an assertive appearance, while the women appear limp and timid. The single image reflects the viewer's experience of examining the provocative women throughout the collage. An act of voyeurism, or peering into a scene for one's own pleasure. The structure of the collage's grid contributes to the voyeuristic experience of viewing the women for entertainment, as the black lines act as a barrier to isolate each woman's image from the other.

Furthermore, Dalí mirrors the male figure's profile by lining up several photographs of masculine-appearing ears throughout the image. The inclusion of the ears symbolizes the patriarchy's role in the diagnosis and treatment of hysteria-stricken patients, as they listened to the patients' hysteric cries and used talk therapy as the cure. The viewer becomes the psychoanalyst for each ecstatic woman, the spectator of the mentally ill. Dalí's collage exemplifies the ways in which Freud's theories justified Surrealism's ideology of female inferiority.

CONCLUSION

The visual analysis of Dalí's *The Phenomenon of Ecstasy*, provides an illuminating example of the ways in which female subjects in Surrealist photographs are sexualized and objectified, posing as passive muses for the artists' fantasies. From a psychoanalytic feminist perspective, the sexist elements of Freud's psychoanalysis, particularly theories on unconscious drives and psychosexual development derived from hysteria research, had a substantial influence on Surrealist artists' portrayal of women. Breton advocated for Freud's theories as the basis of Surrealism, encouraging the artists to explore their existence through psychoanalytic means. As the movement was already rooted in misogyny, Freud's medical status and published works functioned as both an inspiration and justification for the artists to derogatorily portray women.

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NOTES

1. Sigmund Freud and A. A. Brill, *The History of the Psychoanalytic Movement* (New York: Nervous and Mental Disease Pub. Co., 1917), xi.
2. Julien Bogousslavsky and Sebastian Dieguez, "Sigmund Freud and Hysteria: The Etiology of Psychoanalysis," in *Hysteria: The Rise of an Enigma*, vol. 35 (Switzerland: Karger Publishers, 2014), 110.
3. *ibid.*, 111.
4. Georges Didi-Huberman, "Attacks and Exposure," in *Invention of Hysteria: Charcot and the Photographic Iconography of the Salpêtrière*, trans. Alisa Hartz (Cambridge: MIT Press, 1982), 161.
5. Freud and Brill 1917, xii.
6. Hal Foster, *Compulsive Beauty* (Cambridge: MIT Press, 1993), 17.
7. Sigmund Freud, "The Dissolution of the Oedipus Complex," in *The Standard Edition of the Complete Psychological Works of Sigmund Freud*, eds. James Strachey, Anna Freud, and Carrie Lee Rothgeb (London: Hogarth Press, 1964), 174.
8. *ibid.*, 174.
9. *Ibid.*, 178.

10. *ibid.*, 176.
11. Aaron H. Esman, "Psychoanalysis and Surrealism: André Breton and Sigmund Freud," *Journal of the American Psychoanalytic Association* 59, no. 1 (2011): 173.
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13. Alastair Bonnett. "Art, Ideology, and Everyday Space: Subversive Tendencies from Dada to Postmodernism," *Environment and Planning D: Society and Space* 10, no. 1 (1992): 72.
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22. Kuenzli, 1990, 19.
23. *ibid.*
24. *ibid.*
25. Lomas 2000, 53.
26. *ibid.*
27. *ibid.*

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Personality and GPA: The Predictive Roles of Academic Identity and College-Going Culture

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ABSTRACT

Social psychology has established a theoretical relationship between personality and academic performance, but it has yet to identify the process by which personality influences real-world outcomes, such as grade point average. This paper proposes a model that explicates academic identity's role as a mediator in the relationship between the Big Five Factors of personality and college GPA. Specifically, the current paper focuses on the ability of personality to predict academic identity. A college-going culture, or the extent to which a student's high school cultivates a pro-college environment, is hypothesized to moderate the relationship between personality and academic identity. To investigate the hypothesis, self-report measures related to personality and academics were administered to 370 university students. Results generally supported the model, suggesting a process by which students' personalities effect their academic attitudes. Educators are encouraged to foster college-going cultures which they can use to help students who are predisposed to adopt harmful academic identities.

Keywords: academic achievement, academic identity status, big five, college-going culture, personality



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INTRODUCTION

Personality has been shown to predict a variety of outcomes and behaviors, such as workplace performance (Barrick & Mount, 1991) and academic effort (Nofle & Robbins, 2007). However, there are inconsistencies in the literature explaining the relationship between personality and college grade point average. This paper argues against a direct relationship between personality and GPA and instead suggests a relationship dependent on third variables, such as college-going culture, academic identity, and academic behaviors and attitudes (see *Figure 1*). This model is not meant to identify which individuals are doomed for academic failure or destined for academic success. Rather, it serves to identify which students may be predisposed to develop mindsets that will hinder their abilities to succeed. Ultimately, this paper hopes to establish the influence of college-going culture on academic identity formation and subsequently encourage educators to support and foster college-going cultures in high schools.

The Big Five

The Big Five Factor theory proposes five basic trait factors that serve as the building blocks of personality (Costa & McCrae, 1992; Digman, 1990; McCrae & Costa, 1987). These five factors are openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism. Since its inception, the Big Five Factor model has been the focus of many hypotheses hoping to shed light on personality as a predictor of behavior and performance.

Certain personality factors, such as conscientiousness and openness, have been found to consistently predict certain measurements of achievement, such as job performance (Barrick & Mount, 1991) and SAT scores (Nofle & Robbins, 2007). On the other hand, the Big Five Factors only inconsistently predict the outcomes and behaviors that compose academic achievement, such as grade point average, exam scores, and academic effort.

For example, while the argument in favor of a relationship between conscientiousness and student GPA is strong, the predictive power of the other four factors is typically insignificant (Bauer & Liang, 2003; Chamorro-Premuzic & Furnham, 2003; Langford, 2003). In addition, the inability of conscientiousness to consistently predict GPA has led multiple researchers to assume that the relationship between conscientiousness and academic performance may be either partly or completely indirect in nature (Bauer & Liang, 2003; O'Connor & Paunonen, 2007). Academic effort (Nofle & Robbins, 2007) and learning styles (Komarraju, Karau, Schmeck, & Avdic, 2011) are two examples of proposed mediators between the Big Five Factors and GPA. However, while Nofle and Robbins (2007) found weak, but significant mediated correlations between most of the five factors and GPA, these findings failed to replicate in the four other samples analyzed in the same study. Still, the recent shift toward a third-variable model seems probable in explaining this relationship. The current paper considers multiple third variables, including

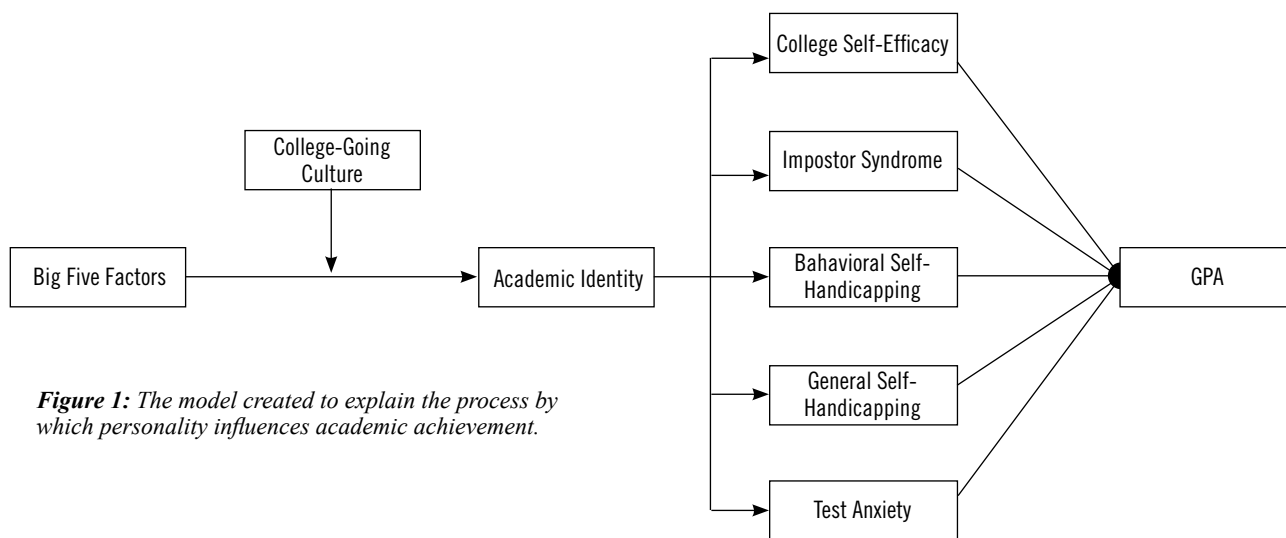


Figure 1: The model created to explain the process by which personality influences academic achievement.

academic identity status and college-going culture, that personality is proposed to predict.

Academic Identity Status

Academic Identity Status (Was, Al-Harthy, Stack-Oden, & Isaacson, 2009; Was & Isaacson, 2008) unites the concepts of identity status and achievement orientation into a singular construct capable of predicting a college student's academic goals, behaviors, and self-concept. Drawn from previous work by Erikson (1963), academic identity status development is dependent on the dimensions of crisis and commitment. The crisis dimension is dependent on the degree of exploration experienced by the student before commitment occurs. The four academic identity statuses align with the identity statuses proposed by Marcia (1966): identity achievement, identity foreclosure, identity moratorium, and identity diffusion. Identity achievement is attained when there is sufficient exploration prior to the commitment, whereas identity foreclosure occurs if a student does not partake in sufficient exploration prior to commitment. Identity foreclosure in college students typically results from a premature commitment brought about by pressure from outside forces, such as pressure from parents to attend college (Was, Al-Harthy, Stack-Oden, & Isaacson, 2009). Next, identity moratorium results from high exploration without commitment. Finally, identity diffusion results from low exploration and low commitment. Similar to the third-variable models proposed by Nofle and Robbins (2007) and Komarraju, Karau, Schmeck, and Avdic (2011), the present paper identifies academic identity status as the third variable needed to identify a reliable path by which personality may influence academic achievement.

College-Going Culture

The present paper proposes that college-going culture moderates the effect of personality on academic identity status. In general, a college-going culture is characterized by pro-college assistance and encouragement from teachers, parents, advisors, and peers at the high school level (Oakes, Mendoza, & Silver, 2004). High schools students exposed to a college-going culture are more likely to attend four-year universities due to the high degrees of social support and personalized attention resulting from the culture (Holland & Farmer-Hinton, 2009). Therefore,

advisors, teachers, parents, and peers are considered key to the effectiveness of a college-going culture. A high school student's social support network is a necessary source of college-related information and emotional assistance (Schneider, 2007). In addition, college-going cultures emphasize the role of the teacher in the student's understanding and preparation for the more challenging college work environment (Schneider, 2007). Rigorous courses and tests are encouraged as a way of preparing students for their general college entrance exams, the Scholastic Aptitude Test (SAT), and college courses. Along with college preparation, college awareness and college eligibility are key components of college readiness (Baker, Clay, & Gratama, 2005). On the path to achieving college readiness, students require a vast amount of college-related information and classroom preparation. For example, students need help understanding the college application process. Information regarding which colleges particular students are eligible to attend, and how to pick a good college match to meet specific student's needs, should be available.

Current Research

Previous literature has established that personality is predictive of grade point average, but the specifics of the relationship are currently unknown. More recent studies have suggested that the predictive power of personality for GPA is at least somewhat dependent on a third variable(s). Therefore, the first half of the present model focuses on college-going culture as just such a third variable, or moderator, for the relationship between personality and academic identity status, and the second half of the model focuses on the relationship between academic identity status, academic behaviors and attitudes, and GPA. The current paper tested the following hypotheses.

Hypothesis 1 states that the Big Five Factors of personality will predict academic identity status. Each personality factor is characterized by a set of unique attitudes that may lead to the inhibition or promotion of exploration and commitment. For example, individuals who are high in openness to experience are more likely to possess a curious nature and individuals high in extraversion are more likely to seek out new experiences (Costa & McCrae, 1992). Such characteristics are reasonably expected to

promote exploration. Therefore, openness and extraversion are predicted to possess a stronger influence on identity statuses with high degrees of exploration. Similarly, high *agreeableness* is typically associated with naivety and submissiveness, which may predict identity statuses with higher degrees of commitment due to an inability or lack of desire to fight against social pressures. The attitudes associated with neuroticism, such as anxiety and frustration, also may lend to the development of non-achieved academic identity statuses. *Conscientiousness* is expected to predict identity achievement due to its association with dedication and carefulness.

Hypothesis 2 states that college-going culture will moderate the strength of the relationship between personality and academic identity status. College-going culture exposes students to information and options related to colleges. For better or worse, the culture also pushes students to commit to four-year colleges immediately following high school. Therefore, it is proposed that greater accessibility to information and encouragement from others to pursue higher education will make students more likely to form an achieved academic identity status. Again, due to the feelings of frustration and anxiety associated with neuroticism, it is expected that students who score high on neuroticism will benefit the most from college-going culture. This effect is expected given the key aspects of college-going culture, including improved access to resources and increased guidance and encouragement from support systems. Therefore, those testing high in neuroticism are expected to become more likely to form an achieved academic identity following exposure to the college-going culture. Therefore, college-going culture is also proposed as a vital point of intervention for students who may be predisposed to adopt a non-achievement academic identity.

METHODS

Participants

The participants were 370 undergraduate students attending the same university in Southern California. Of the 370 participants, 65% of participants (237) indicated they were female, 35% of participants (132) indicated they were male, and one participant declined to indicate a gender. The average age was 19.00 (*Min* = 17; *Max* = 28; *SD* = 1.37). The ethnically diverse sample consisted of 41.4% Asian

American students, 32.7% Hispanic/Latino students, 8.4% Caucasians students, 5.4% African Americans students, and 10.6% who either indicated a Mixed Heritage or chose the option of Other.

Of the students participating in the study, 75.6% of participants were completing their first or second year of university education (*first-year* = 185, *second-year* = 95, *third-year* = 61, *fourth-year* = 29). In terms of academic major, 37.3% of participants (138) were pursuing a degree in the College of Humanities, Arts, or Social Sciences, whereas 57.6% of participants (213) were pursuing a degree in the College of Natural Sciences or the College of Engineering. 4.6% of participants reported an undeclared or undecided academic major.

Materials

Big Five Personality Factors. The Big Five Inventory (John & Srivastava, 1999) was administered to assess the extent to which participants expressed certain personality traits. The Big Five Inventory (BFI) is a 44-item instrument with a 5-point Likert scale. The scale ranged from 1 (Disagree Strongly) to 5 (Agree Strongly). The measure has five subscales corresponding to each of the Big Five Factors: *openness*, *conscientiousness*, *extraversion*, *agreeableness*, and *neuroticism*. Participants were instructed to rate the extent to which they identified as someone who exhibits certain characteristics or beliefs. Each phrase begins with “I see myself as someone who...” and is followed by 44 statements comprising the elements of the subscales. The openness subscale includes items such as “likes to reflect, play with ideas”. The conscientiousness subscale includes items such as “is a reliable worker”. The extraversion subscale includes items such as “is outgoing, sociable”. The agreeableness subscale includes items such as “has a forgiving nature”. The neuroticism subscales include items such as “gets nervous easily”. Cronbach’s alpha was used to determine the degree of reliability and agreement of the items within each subscale; openness = 0.74, conscientiousness = 0.82, extraversion = 0.86, agreeableness = 0.74, and neuroticism = 0.78.

College-Going Culture. An adaptation of the College-Going Culture questionnaire (Oakes, Mendoza, & Silver, 2004) was administered to determine the amount of college-

related support and resources students received in high school. The College-Going Culture questionnaire (CGC) is a 26-item instrument with a 4-point Likert scale. The scale ranged from 1 (Strongly Disagree) to 4 (Strongly Agree). Examples of items from the CGC include statement such as: “College representatives regularly visited your campus to speak with staff and students;” “Your high school offered counseling regarding courses that would prepare you for a four-year college;” and “Your family prepared you to go to college”. Cronbach’s alpha for the questionnaire = 0.85, indicating a high degree of reliability and item agreement.

Academic Identity Status. The Academic Identity Measure (Was & Isaacson, 2008) was administered to assess the central academic attitudes of the participants. The Academic Identity Measure (AIM) is a 40-item instrument with a 5-point Likert scale. The Likert scale ranged from 1 (Not at all like me) to 4 (Very much like me). The measure consists of four subscales: achieved, diffused, foreclosed, and moratorium. Each subscale represents one of the four potential academic identities by the same name. The achieved subscale includes items such as “I know why I am in college and have clear goals I want to achieve”. The diffused subscale includes items such as “I don’t have clear priorities for school and life. I usually just go with the flow”. The foreclosed subscale includes items such as “If I had to pay for my own education I probably wouldn’t even be in school even if I had the money”. The moratorium subscale includes items such as “My view of grades and studying fluctuates: sometimes I am conscientious, other times I’m lazy”. Cronbach’s alpha for each subscale is as follows: achieved = 0.76, diffused = 0.76, foreclosed = 0.77, and moratorium = 0.85.

Procedure

Students were given the option to participate in the study and receive course credit as compensation. The study was presented as a survey of student beliefs regarding college success. Students who agreed to participate were required to report to a reserved computer lab on the university’s campus. The controlled environment of the reserved computer lab minimized distractions and potential variances in experience during data collection.

Before beginning the digital survey, participants were

instructed to read and sign the informed consent form. Next, each participant was assigned a unique identification number and a personal desktop computer to allow him or her to access the survey. The digital survey began with a demographic questionnaire and then it continued with multiple self-report measures. The demographic questionnaire included items that solicited non-academic information (e.g. gender, age, and ethnicity) and academic information (e.g. academic concentration and college generation status). The self-report measures included the Big Five Inventory, the College-Going Culture Questionnaire, and the Academic Identity Measure. Participants were allowed up to one hour to finish the survey. Each computer was reset following each session.

RESULTS

Simple moderated regression analyses were conducted to determine the degree to which college-going culture influences the effect of personality on the formation of academic identity status. The results of the moderated regression analyses are summarized in Table 1. Aside from three exceptions, each of the five personality factors holds significant predictive value for each of the four academic identity statuses. College-going culture moderated three of these relationships: openness to experience and foreclosure, conscientiousness and moratorium, and neuroticism and foreclosure. Specifically, when college-going culture was included, the relationship between openness and foreclosure changed from negative to positive, with the standardized coefficient (β) increasing from $\beta = -.15$ to $\beta = .18$, $F(3, 337) = 12.35$, $p < 0.01$. Similarly, the relationship between conscientiousness and moratorium weakened, $\beta = -.46$ to $\beta = -.16$, $F(3, 329) = 32.85$, $p < 0.01$. The relationship between neuroticism and foreclosure changed from positive to negative and was reduced from $\beta = .24$ to $\beta = -.10$, $F(3, 337) = 14.85$, $p < 0.05$.

The results also revealed that college-going culture is more likely to independently influence academic identity than it is to influence academic identity status via a significant interaction with personality. College-going culture consistently predicted an increased likelihood of identity achievement and identity foreclosure, even when a personality factor was negatively associated with or had no significant association with identity status.

DISCUSSION

To properly understand the relationship between personality and GPA, the present paper suggested the importance of college-going culture and academic identity status as third variables. Therefore, the relationship between personality, college-going culture, and the Big Five Factors was analyzed. Regression analyses provided support for Hypothesis 1. Hypothesis 2 was not generally supported. Instead, analyses revealed a direct relationship between college-going culture and academic identity.

As shown by *Table 1*, the results supported Hypothesis 1. There is a significant relationship between the Big Five Factors and academic identity status. Openness is strongly predictive of an achieved academic identity and negatively related to the non-achieved identities. Conscientiousness, agreeableness, and extraversion are also positively associated with identity achievement and negatively associated with non-achievement identities. Notably, conscientiousness is not significantly predictive of identity

foreclosure, but it has a strong negative association with moratorium and diffusion. In other words, while being highly conscientious does not predict a foreclosed identity, it may still buffer against academic identity statuses that are low in commitment. This may be due to the traits of determination and hard work that are associated with conscientiousness (Costa & McCrae, 1992). As predicted, neuroticism is also most significantly associated with non-achievement identities, especially foreclosure and moratorium. These results suggest that students who score high on neuroticism are less likely to form an achieved academic identity, potentially due to the traits of anxiety and frustration (Costa & McCrae, 1992).

Regarding Hypothesis 2, there was little support for the moderation effect of college-going culture on personality’s predictive value of academic identity status. College-going culture reduces the predictive strength of conscientiousness on identity moratorium. However, the association still remains negative. In addition, individuals who score high

Identity Factor	Personality Predictor	Beta (BF)	t-value (BF)	Beta (CGC)	t-value (CGC)	Beta (CGCxBF)	t-value (CGCxBF)	R ²	Adjusted R ²
Achieved	Openness	0.24***	4.34	0.15**	2.75	0.07	0.27	0.09	0.08
	Conscientiousness	0.44***	9.05	0.09	1.84	0.04	0.73	0.23	0.21
	Extraversion	0.17**	3.16	0.16**	2.84	-0.02	-0.35	0.06	0.05
	Agreeableness	0.21***	3.98	0.15**	2.67	0.06	1.15	0.08	0.07
	Neuroticism	-0.11*	-2.03	0.16**	2.96	-0.01	-0.23	0.04	0.03
Foreclosed	Openness	-0.15**	-2.94	0.22***	4.20	0.18**	3.26	0.10	0.09
	Conscientiousness	-0.01	-0.25	0.23***	4.30	0.03	0.61	0.05	0.04
	Extraversion	-0.11*	-2.13	0.24***	4.42	0.03	0.58	0.06	0.06
	Agreeableness	0.05	0.91	0.22***	4.12	0.01	0.19	0.05	0.05
	Neuroticism	0.24***	4.63	0.24***	4.69	-0.10*	-2.04	0.17	0.11
Moratorium	Openness	-0.08	-1.49	-0.01	-0.21	-0.02	-0.33	0.01	-0.00
	Conscientiousness	-0.46***	-9.27	0.05	1.01	-0.16**	-3.24	0.23	0.22
	Extraversion	-0.14*	-2.49	-0.00	-0.06	-0.01	-0.14	0.02	0.01
	Agreeableness	-0.18***	-3.37	0.00	0.04	-0.04	-0.86	0.04	0.03
	Neuroticism	0.25***	4.66	0.00	0.06	0.05	0.93	0.07	0.06
Diffused	Openness	-0.14**	-2.62	-0.02	-0.40	-0.01	-0.22	0.02	0.01
	Conscientiousness	-0.48***	-10.00	0.04	0.83	-0.22	-0.47	0.23	0.23
	Extraversion	-0.12*	-2.22	-0.02	-0.30	-0.06	-1.02	0.02	0.01
	Agreeableness	-0.19***	-3.64	-0.01	-0.23	-0.04	-0.75	0.04	0.03
	Neuroticism	0.14*	2.50	-0.02	-0.27	0.06	1.10	0.02	0.01

Table 1: Moderated regression analyses with Big Five personality traits and college-going climate regressed on academic identity status. Note: CGC = College-Going Culture; BF = Big Five. * p < .05; ** p < .01; *** p < .001.

in openness to experience become more likely to adopt a foreclosed identity when exposed to a college-going culture. These results might seem to suggest that college-going culture may have a harmful effect on students' academic identity formation. However, college-going culture also reduces the predictive strength of neuroticism on foreclosure to a degree that changes the nature of the relationship from concurrent to oppositional. Perhaps the frustration and anxiety surrounding the college selection process is diminished by the exploration factor of college-going culture. This finding supports the use of college-going culture to assist students predisposed to harmful academic identities.

To expand upon Hypothesis 2, the results revealed an unexpected relationship between college-going culture and academic identity that is independent of personality. Perhaps then college-going culture's variable influence on personality is due to the latter's relatively stable nature (McCrae & Costa, 1987) and not a signal of ineffectiveness. Indeed, college-going culture consistently and strongly predicts identity achievement and identity foreclosure. These findings coincide with college-going culture's emphasis on college attendance (Holland & Farmer-Hinton, 2009) and the high degrees of commitment seen in identity achievement and foreclosure (Was & Isaacson,

2008). Greater integration of exploration into college-going culture, which already exists in the form of college information and advising (Baker, Clay, & Gratama, 2005), will possibly reduce instances of identity foreclosure and promote greater identity achievement.

The results also support previous research that has called for the consideration of third variables in the relationship between personality and GPA (Bauer & Liang, 2003; O'Connor & Paunonen, 2007). Future research is encouraged to advance the generalizability of this particular third-variable model by drawing from populations representative of students who are in their last years of high school and their first years in college. The validation of academic identity status as the missing link between personality and GPA creates a promising opportunity for academic intervention. Academic identity status, unlike the Big Five Factors of personality, is not a construct resistant to guidance and influence from outside factors. College-going culture is a strong and reliable predictor of academic identity achievement. In addition, these analyses reveal that despite the stubborn nature of personality, students who score high on neuroticism still benefit largely from exposure to college-going culture. Therefore, high school educators are encouraged to adopt college-going cultures to foster beneficial academic identities in their students.

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Building a Sense of Self: The Link between Emotion Regulation and Self-Esteem in Young Adults

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ABSTRACT

Emotion regulation is the process through which a person changes his or her emotions. Individuals may change their emotions in many ways, and these different aspects of emotion regulation might have different implications for one's self-esteem. Self-esteem is defined as an individual's concept of the self. Despite the substantial research on these topics, there has been a lack of research on the links between emotion regulation and self-esteem. The present study aimed to explore the link between emotion regulation and self-esteem in young adults, as well as to examine potential gender differences in this association. Based on current research, we predicted that men would have higher self-esteem than women, whereas women would have a stronger capacity to regulate their emotions. Furthermore, we predicted women would show a stronger association between emotion regulation and self-esteem. Participants were asked to answer the Difficulties in Emotion Regulation (DERS) questionnaire as well as a singular measure to assess their self-esteem. The results of the present study were consistent with our hypothesis that men would have a higher self-esteem, and that women would show a stronger association between emotion regulation and self-esteem. Our study adds to a growing body of research on the importance of emotion regulation for self-esteem.

Keywords: Emotion Regulation, Self-Esteem, Gender Differences, Difficulties in Emotion Regulation, Young adults, Self-Concept



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Dr. Elizabeth Davis is an Assistant Professor in the Psychology Department at UC Riverside. She earned her PhD in Developmental Psychology from the University of California, Irvine in 2009. Research in the Emotion Regulation Lab focuses on understanding how emotion regulation relates to adaptive outcomes (e.g., learning) and maladaptive outcomes (e.g., anxiety) in childhood. Emotion regulation can be broadly defined as the set of processes by which people influence the timing, expression, and experience of their emotions. The lab's work to date has aimed 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 whether they can regulate emotion effectively. This research also focuses on identifying mechanisms responsible for effective emotion regulation (e.g., attentional focus) to explain why certain emotion regulation strategies attenuate negative emotion and distress. Ultimately, this program of research can be viewed as providing an empirical basis for interventions aimed at improving children's emotion regulation abilities and mitigating risk for maladaptive outcomes.

INTRODUCTION

The transition from adolescence to adulthood can be both an enjoyable and a challenging period in a young adult's lifetime. These changes can bring about new emotions, perspectives, and sense of self as a young adult begins to create a new outlook on the world. As obstacles and hardships are encountered, it is frequently necessary for people to regulate, modify, or change the emotions that are generated by these events. Emotion regulation involves the use of behavioral, cognitive, emotional, and attentional tools to change or maintain the experience and expression of emotions (Brody & Hall, 2009). Regulating emotions is a process that most people engage with on a daily basis. The stress someone may feel during a job interview or when managing the constant demands of homework and course preparation can be regulated effectively through breathing exercises and meditation. Even during moments of hopelessness and sentiments of despair, the process of changing emotions enables people to feel less emotional exhaustion and more life satisfaction (Hülshager, Alberts, Feinholdt, & Lang, 2013). The current study aims to identify individual and gender variations in emotion regulation during periods of distress and unpleasant events.

There is substantial evidence that women and men regulate their emotions in different ways. For example, men have been found to use cognitive reappraisal (i.e., reframing the negative event in a less emotional way) less effectively than women (McRae, Ochsner, & Mauss, 2008). Men have also been found to suppress their anger and depressive feelings more often than women (Kwon, Yoon, Joormann, & Kwon, 2013). On the other hand, women are more likely to use rumination (i.e., repetitively pondering about the situation), which in turn can lead to heightened feelings of anxiety and inadequacy over the present situation. (Kwon, Yoon, Joormann, & Kwon, 2013).

Gender differences have also been found in self-esteem. Self-esteem is defined as a person's general sense of worth (Bajaj, Gupta, & Pande, 2016). An individual's sense of self plays a vital role in one's identity. A low self-esteem can generate sentiments of inadequacy or fulfillment during moments of high stress, such as in the case of adopting a set of duties through a new job position or when awaiting a medical diagnosis. Research has also suggested that men

tend to have higher self-esteem than women (Zuckerman, Li, & Hall, 2016). Although men may report having higher self-esteem than women, a person's self-esteem does not fully develop until about age 18. Therefore, gender differences in self-esteem do not appear to exist until later in adolescence (Zuckerman, Li, & Hall, 2016). In essence, young adult men may report having higher self-esteem than women, but ultimately societal expectations and sociocultural factors mold these perceptions of the self.

Self-esteem plays a vital role in enhancing one's well-being and can reduce negative affect (Bajaj, Gupta, & Pande, 2016). Therefore, it is not surprising that there seems to be a link between emotion regulation and self-esteem. Specifically, more adaptive emotion regulation appears to act as a buffer for low self-esteem (Bajaj, Gupta, & Pande, 2016). Although there is some evidence that general measures of emotion regulation are associated with self-esteem, less is known about how specific aspects of emotion regulation might relate to self-esteem. Moreover, considering that there seem to be gender differences in both self-esteem and emotion regulation in early adulthood, more research is needed to understand if the link between self-esteem and emotion regulation differs between men and women.

Current Study

Several studies within the past decade have indicated that there is a relation between emotion regulation and self-esteem (Bajaj, Gupta, & Pande, 2016). Past research, however, has overlooked the link between specific aspects of emotion regulation, such as access to emotion regulation strategies, as a predictor of self-esteem. There is evidence that men and women differ in how they regulate their emotions (Kwon, Yoon, Joormann, & Kwon, 2013). If this is true, then gender differences in emotion regulation should also be reflected in the link between emotion regulation and self-esteem. The present study aimed to assess whether differences in various aspects of emotion regulation relates to self-esteem in young adults. Additionally, we examined the role of gender in further contextualizing the hypothesized relation between emotion regulation and self-esteem. Based on previous studies, we hypothesized that men would show higher self-esteem than women, and that women would show better emotion regulation than

men. When looking at the link between emotion regulation and self-esteem, we expected the relation between the constructs to be stronger for women compared to men.

METHOD

Participants

Participants included 91 young adults ($M_{age} = 19.41$ years, $SD = 1.56$; 71 women) who participated in the study in exchange for research credits for a course. Racial and ethnic distribution varied, 41% endorsed being Asian, 17% endorsed being Hispanic, 13% endorsed being white/Caucasian, 7% endorsed more than one ethnicity, 5% endorsed being part of an ethnic group not specified above, 2% endorsed being black, and 15% did not report race/ethnicity.

Procedure

Participants came to the Emotion Regulation Lab at the University of California Riverside for a two-hour single session study. Informed consent was obtained from all participants before the study started. Participants completed computer tasks and interviews (not considered here) and were asked to report on their gender, their self-esteem, their emotion regulation, as well as on other family and personal characteristics. At the end of the study, participants were debriefed and received research credit for their participation. Procedures were completed in English.

Stimuli and Measures

Self-Esteem. We assessed self-esteem with a single item. Participants were asked to indicate using a 7-point scale (1 = strongly disagree; 7 = strongly agree) how much they agreed with the statement “I have high self-esteem.”

Emotion Regulation. Participants completed the *Difficulties in Emotion Regulation Scale (DERS)*. This 36-item questionnaire asked participants to indicate how often the statements applied to them using a scale of 1 to 5 (1 = almost never; 5 = almost always). The questionnaire included questions such as, “I am clear about my feelings,” and, “I pay attention to how I feel.” The present study utilized all six subscales from the DERS questionnaire. The *Nonacceptance* subscale measures the extent to which a person has a negative reaction to their own distress. The *Goals* subscale measures difficulty in focusing and completing tasks when negative emotions are present.

The *Impulse* subscale detects the difficulty an individual may have in controlling their behavior while experiencing negative emotions. The *Awareness* subscale provides an indication of a person’s negligence and disregard for his or her emotions. The *Strategies* subscale illustrates an individual’s belief in his or her capability to regulate their emotions. Similarly, the *Clarity* subscale reflects an individual’s capacity to cultivate a clear understanding of their emotions. Reliability for all subscales was good in this sample ($\alpha = .79-.88$).

RESULTS

The present study aimed to answer the question of whether different aspects of emotion regulation are linked to self-esteem, and whether there were gender differences in the link between emotion regulation and self-esteem.

Gender differences in self-esteem: As expected, there was a significant gender difference in self-esteem, $t(88) = 2.669$, $p = .008$. Men ($M = 5.21$, $SD = 1.34$) reported having higher self-esteem than did women ($M = 4.39$, $SD = 1.34$).

Gender differences in emotion regulation: In contrast to expectations, there were no significant gender differences in emotion regulation. Men and women did not differ in terms of nonacceptance, $t(89) = .595$, $p = .553$; goals, $t(89) = -1.089$, $p = .279$; strategies, $t(89) = -.235$, $p = .815$; impulse, $t(89) = 1.212$, $p = .229$; awareness, $t(89) = .834$, $p = .407$ or clarity, $t(89) = 1.406$, $p = .163$.

Is emotion regulation associated with self-esteem? Self-esteem was negatively associated with nonacceptance ($r = -.299$, $p = 0.004$), therefore indicating that having a stronger typical negative reaction to one’s own distress and emotions was associated with lower self-esteem. Furthermore, the DERS goals scale ($r = -.299$, $p = .004$) correlated with self-esteem, indicating that greater difficulty in focusing and accomplishing tasks was also associated with lower self-esteem. Self-esteem was significantly associated with DERS clarity ($r = -.396$, $p < .001$) such that greater difficulties in acknowledging and creating a clear understanding of one’s emotions were associated with lower self-esteem. Lastly, self-esteem was also significantly negatively associated with DERS strategies ($r = -.351$, $p = .001$) such that greater difficulties finding

strategies to change negative emotions was associated with lower self-esteem. Thus, though self-esteem was not related to all six DERS subscales, our hypotheses about the relation between emotion regulation and self-esteem were largely supported by these correlational findings (*Table 1*).

Are there gender differences in these patterns of associations? Although we did not find significant group differences between men and women for any of the emotion regulation variables, one of our main goals was to explore how the link between emotion regulation and self-esteem might differ by gender. Thus, we still conducted our planned correlations for women and men separately to explore these potential differences in the relations between emotion regulation and self-esteem.

Men. Self-esteem was significantly associated with DERS clarity for men ($r = -.495, p = .007$), showing the same pattern as the correlation with the whole sample. The correlation between DERS strategies and self-esteem was significant ($r = -.447, p = .017$) with greater difficulties being associated with lower self-esteem. Lastly, the correlation between DERS goals and self-esteem was also significant ($r = -.401, p = .034$) showing that having difficulty in completing tasks was associated with having a lower self-esteem for men.

Women. Self-esteem was significantly associated with DERS clarity for women ($r = -.435, p < .001$) showing the same patterns as the correlation with the whole sample. A similar pattern emerged for DERS strategies ($r = -.324, p = .011$) with greater difficulties being associated with lower self-esteem. The correlation between self-esteem and DERS nonacceptance scale was also significant ($r = -.339, p = .007$), suggesting

that non-acceptance of one’s emotions was associated with a lower self-esteem. Lastly, a greater inability to acknowledge one’s emotions (i.e., DERS awareness) was associated with lower self-esteem ($r = -.338, p = .008$).

Differences in the magnitude of the relation between emotion regulation and self-esteem for women and men. We used Fisher’s r to z transformations to assess potential magnitude differences in the correlations between DERS strategies and clarity and self-esteem for women and men, because these were the only correlations that were significant for both men and women. Results suggest that the magnitude of these correlations did not significantly differ between men and women for DERS strategies ($z = -.061, p = .542$) or for DERS clarity ($z = -.32, p = .749$), in contrast to our expectations.

DISCUSSION

The goal of the present study was to assess associations between various aspects of emotion regulation and self-esteem, and to examine potential gender differences in these associations. Studying self-esteem is vital, as high self-esteem has been associated with better coping mechanisms and setting higher standards for one’s self (Baumeister, Campbell, Krueger, & Vohs, 2003). In addition, low self-esteem has been associated with greater aggressive behavior (Donnellan, Trzesniewski, Robins, Moffitt, & Caspi, 2005), underscoring the importance of understanding how emotion regulation and self-esteem are related for men and women. Thus, we aimed to further look at potential gender differences in self-esteem by exploring how emotion regulation might differentially relate to self-esteem for men and women. We hypothesized that men would have higher self-esteem, whereas women would show better emotion regulation. Furthermore, we hypothesized that women would show a stronger association between emotion regulation ability and their self-esteem. Our findings showed that young adult men did in fact report significantly higher self-esteem in comparison to young adult women. However, in contrast with our hypothesis, there were no significant gender differences in emotion regulation. Despite the lack of group level differences in emotion regulation, the correlations between various facets of emotion regulation and self-esteem revealed that there were differences in how emotion regulation was linked to self-esteem for men and women. Specifically, when

DERS Subscales	Everyone	Men	Women
DERS Goals Scale	-.299**	-.401*	-.235
DERS Non-Acceptance	-.299**	-.310	-.339**
DERS Awareness	-.208*	.012	-.338**
DERS Strategies	-.351**	-.447*	-.324*
DERS Clarity	-.396**	-.495*	-.435**
DERS Impulse	-.206	-.374	-.195

*Table 1. Correlations between self-esteem and the emotion regulation subscales. + $p < .10$; * $p < .05$; ** $p < .01$*

looking at the correlations by gender the clarity, strategies, and goals subscales were significantly correlated with self-esteem for men, whereas for women, self-esteem was significantly associated with nonacceptance, awareness, strategies, and clarity. Thus, these findings partially support our hypothesis that a stronger link between aspects of emotion regulation and self-esteem would be seen among women compared to men.

There are several possible explanations as to why young adult men may have higher self-esteem than their female counterparts. It is possible that societal stereotypes, that define men as less emotional in comparison to women, may introduce psychological stress that men feel the need to fulfill and surpass (Kling, Hyde, Shibley, Buswell, Showers, 1999). The significant associations for men (clarity, strategies, and goals) suggest that difficulties understanding their emotions, difficulties accessing strategies to change how they feel, and difficulty focusing and completing the task at hand because of these negative emotions, are particularly important for understanding men's self-esteem. Difficulties in these specific aspects of emotion regulation may present a threat to masculine identity and self-esteem (Tager, Good, Brammer, 2010). Moreover, the norms established by sociocultural factors can further impede on a male's sense of self and ability to acknowledge their emotions.

Gender differences in self-esteem are noticeable from early adolescence, more specifically beginning at the age of 15 (Zuckerman, Li, & Hall, 2016). During this time, girls are found to have lower self-esteem than boys, as girls become more exposed to the media presentation of women and girls as passive and are exposed to the societal standards of body image (Kling, Hyde, Showers, Buswell, 1999). Participants in the present study were young adults, therefore it is possible that female participants are not only continuously in the process of developing their identity but are also presenting differences in self-concept in comparison to men that would be expected based on these societal factors. The primary difference between the correlation of emotion regulation and self-esteem in both genders was that low self-esteem in women was associated with more and different aspects of emotion regulation, especially problems accepting their emotions and acknowledging them. It is possible that women's consistent exposure to passive perceptions of women and the unfeasible

criteria for body thinness that is predominant in contemporary western societies such as the United States, results in women more frequently experiencing negative situations that can directly undermine their self-esteem more often. This in turn may provide an explanation as to why emotion regulation appears to be more strongly associated to self-esteem in women compared to men; however more research is needed on this particular topic.

The findings of the present study are tempered by some limitations. For example, we used a single question to measure self-esteem, which may not account for other aspects of self-esteem and self-concept that differentially relate to emotion regulation. Another limitation is that most of our sample were women, and the relative underrepresentation of men may have limited the variance in men's emotion regulation and self-esteem we could detect. In addition, we focused on young adults, but research suggests that these links might be changing throughout development, therefore, more research is needed to better understand how these associations might be changing throughout the lifespan.

Although the present study utilized a single question to measure self-esteem, we believe that this was a sufficient measure of self-esteem because of the range of possible responses (1-7). As previous research suggests, self-esteem can be classified as high or low. In essence, an ordinal scale (like the one we used) provides a concise and finite evaluation of participants' perception on their self-esteem. The results in the study did not confirm our hypothesis that women had better emotion regulation than men, however, the results supported our hypothesis that women would have a stronger link between emotion regulation and self-esteem. Therefore, it is possible that having a larger sample size of women in the present study may have provided a more illustrative evaluation of the link between emotion regulation and self-esteem. Current research also suggests that women begin to develop a lower self-esteem during later adolescence. By studying a larger sample of female participants, our study confirms past research on gender variations in self-esteem. Future studies should continue to evaluate the link between emotion regulation and self-esteem by assessing a larger number of men, and by looking at several developmental stages. Exploring this association across adolescence, young adulthood, and middle age may provide useful information about when emotion regulation is

most important for self-esteem. This knowledge, in turn, can be used by clinicians to generate intervention efforts that are most appropriate for the particular developmental stage of the patient.

The current study adds to the growing body of research on

how emotion regulation relates to self-esteem and expands knowledge in this area by highlighting how different aspects of emotion regulation are related to self-esteem for men and women. This is an important first step in clarifying the role and importance of emotion regulation in the cultivation of one's sense of self.

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Parent Psychopathology and Parent-Child Conflict Interact to Predict Children's Anxiety but Not Depression

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ABSTRACT

Correlates of child psychopathology have been studied for decades (Ollendick & Herson, 1989). Research has shown that parental psychopathology can influence child psychopathology through a combination of familial and environmental factors (Biedel & Turner, 1997; Burstein et al., 2010). Parent-child interactions have also been found to relate to child psychopathology (Donenberg & Weisz, 1997). A possible explanation for these relations is that the behavior of parents experiencing psychopathology symptoms differs from parents not facing these difficulties, like displaying more hostile behavior towards their offspring (Burstein et al., 2010). The present study examines the interaction of parent psychopathology and parent-child conflict during a stressful task to see if conflict moderated the relation between parents' and children's psychopathology. We studied whether the relation between parent and child symptoms would be stronger for dyads characterized by conflict. 184 children aged 3 to 11 (91 boys and 93 girls) visited the lab. Parents completed questionnaires to measure their depression and anxiety symptoms, as well as the child's anxiety and depressive symptoms. The parent and the child also participated in a Lego task where instances of child conflict were observed. Results showed that parent anxiety interacted with parent-child conflict to predict children's anxiety symptoms, such that parents' anxiety predicted children's anxiety only among dyads characterized by high levels of conflict. The same was not true for depressive symptoms. The current study expands research about moderators by showing that the link between parent and child psychopathology is qualified by other aspects of the family environment.

Keywords: Psychopathology, Parent-Child Conflict, Anxiety Symptoms, Depressive Symptoms, Internalizing Disorders



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Dr. Elizabeth Davis is an Assistant Professor in the Psychology Department at UC Riverside. She earned her PhD in Developmental Psychology from the University of California, Irvine in 2009. Research in the Emotion Regulation Lab focuses on understanding how emotion regulation relates to adaptive outcomes (e.g., learning) and maladaptive outcomes (e.g., anxiety) in childhood. Emotion regulation can be broadly defined as the set of processes by which people influence the timing, expression, and experience of their emotions. The lab's work to date has aimed 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 whether they can regulate emotion effectively. This research also focuses on identifying mechanisms responsible for effective emotion regulation (e.g., attentional focus) to explain why certain emotion regulation strategies attenuate negative emotion and distress. Ultimately, this program of research can be viewed as providing an empirical basis for interventions aimed at improving children's emotion regulation abilities and mitigating risk for maladaptive outcomes.

INTRODUCTION

Parents are one of the greatest influences on a child's life. Children's development is heavily influenced by both biological and psycho-social aspects of parenting (Ollendick & Herson, 1989). Thus, it is not surprising that parents and children display similar symptoms of psychopathology. A reason why we see this relation between parents' and children's psychopathology may be that children learn, mimic, and internalize their parents' disordered behaviors. Children are at the greatest risk for developing the type of psychopathology demonstrated by their parents because of environmental factors such as parent modeling (Burstein et al., 2010). This is important as the internalization of these behaviors might be a mechanism through which child psychopathology emerges and is maintained throughout the lifespan. Looking at specific factors such as parent psychopathology and parent-child conflict are important when assessing which children are at a higher risk for developing psychopathology. With more research being done on this subject, we are able to minimize the risk factors that increase the likelihood of child psychopathology. Additionally, research on these relations can help create early intervention and prevention measures for child anxiety and depression.

A great amount of research has shown parents' influence on child psychopathology; factors that have been studied vary from parent psychopathology (Biedel & Turner, 1997; Burstein et al., 2010), parent-child interactions (Donenberg & Weisz, 1997; Caron et al., 2006), style of parenting (Van Der Bruggen et al., 2008), and more. First and foremost, parent psychopathology has been found to be a strong predictor for child psychopathology. Researchers have found that children of parents with anxiety disorders and depressive disorders were more likely to have a diagnosable disorder than children of parents that did not have these disorders (Biedel & Turner, 1997). According to this research, the chances of a child having a disorder ranged from 5.05 to 6.25 times higher if the parent had anxiety, depression, or both (Beidel & Turner, 1997). There is a strong link between children's depression and anxiety, and their parent's psychopathology. Therefore, having a parent with a history of psychopathology can affect a child in a multitude of ways.

Parent-child interactions have a strong association with symptoms of depression and anxiety in children (Marmorstein & Iacono, 2004), suggesting that there might be other factors of the parent-child relationship that could account for the emergence of psychopathology in children. One study found that there was an association between child anxiety and parental control (Van Der Bruggen et al., 2008). Interestingly, previous research has associated adult depression/anxiety with reports of over-controlling and dominant behavior (Donenberg & Weisz, 1997). These interactions could lead to parents having more conflict with their children, which in turn can be correlated with symptoms of depression and anxiety. It has been shown that high parent-child conflict was associated with major depression in adolescence (Marmorstein & Iacono, 2004). In this study, we examined instances of parent-child conflict during a stressful task to see if the way parents act in situations like these predicts child symptoms of depression and anxiety when also considering parents' symptoms. Considering these two aspects of a child's environment together is important, as symptoms may be especially pronounced for children who have both a parent with psychopathology and a relationship characterized by conflict with their parents.

Current Study

In the current study, we looked at parent depression and anxiety along with parent-child conflict to see if conflict between parents and their children during a stressful task moderated the effects of parent psychopathology on children's depression and/or anxiety. Based on past work done on the link between parent and child psychopathology, we hypothesized that parent depression and anxiety would be positively correlated with child depression and anxiety. We also hypothesized that parent-child conflict would be positively associated with child psychopathology. Moreover, we were interested in exploring parent-child conflict as a moderator of the link between parent psychopathology and child psychopathology. We expected parent-child conflict to moderate the effect of parent depression on child depression and the effect of parent anxiety on child anxiety, such that parent psychopathology would be a particularly important predictor of child psychopathology for children experiencing high conflict.

METHOD

Participants

Our study included a sample of a total of 184 children, ages 3 to 11 ($M = 7.67$, $SD = 2.30$). This sample included 91 boys and 93 girls. In terms of ethnicity, children were reported by parents as Caucasian (18.2%), African American (10.7%), Hispanic (29.4%), Asian American (2.1%), Other (2.1%), and More than one race (35.3%). Of the caregivers that came in, 153 were mothers and 28 were fathers. Mothers' formal schooling ranged from grade school (1.6%) to a Doctoral degree (2.7%) with the mean formal education level of a trade, technical, or vocational degree ($M = 4.96$, $SD = 1.34$). Fathers' formal schooling ranged from Grade School (1.6%) to a Doctoral degree (3.6%) with the mean also being closer to having a trade, technical, or vocational degree level ($M = 4.77$, $SD = 1.42$). Family income ranged from \$15,000 or less (15.5%) to above \$100,000 (11.8%) with the mean income being in the \$41,000 to \$50,000 bracket ($M = 5.10$, $SD = 3.35$).

Procedure

Families came to the Emotion Regulation lab for one visit. Before any study procedures began, informed consent was obtained from the parents, and assent (verbal and written) was obtained from the children. While children completed a series of engaging tasks (not considered here), parents completed questionnaires about themselves and their child (e.g., demographics, child psychopathology symptoms, and their own symptoms). About half-way through the study, parents were invited to join their child for a series of tasks. Of importance for this study is a frustrating Lego task that they worked on together. Our measure of parent-child conflict was coded from behavior in this task (described below). At the end of the study, families received a small honorarium for their participation and children chose a toy to take home as a thank-you gift. All procedures were done in English.

Stimuli and Measures

Parent-Child conflict. The child and their parent were asked to work on completing a difficult Lego structure together. During the first 5 minutes of the task (Phase 1), parents were given the instruction manual on how to complete the Lego but were asked to only provide verbal help. For the second part of the task (also 5 minutes; Phase

2) parents could physically help their child if they wanted. The Lego structure was a highly complex structure too difficult to complete in 10 minutes even for an adult, thus, the task was designed to elicit frustration for both parent and child. Parent-child conflict was globally coded from this task using a 5-point Likert scale (1 = *low conflict*; 5 = *high conflict*), based on the intensity and duration of child and parents' distress-related or conflict-related behaviors and verbalizations. This could include verbalizations such as "Hey, don't get mad at dad!" or non-verbal behaviors such as throwing Lego pieces, crossing arms, and frowning. Two separate codes were assigned (one for each Phase) but we used the average conflict observed in the two Phases for analyses. Both frequency and intensity of these behaviors and verbalizations were used to assign a level of conflict. Inter-rater reliability was excellent (93% agreement).

Parent psychopathology. For depression, we used the *Center for Epidemiological Studies-Depression* questionnaire (CES-D; Radloff, 1975). The CES-D is a 20-item measure that asks parents how often in the past week have they had various symptoms associated with depression (e.g., restless sleep, poor appetite, feeling lonely). Responses range from 0 to 3 for each question (0 = *Rarely or None of the Time*; 3 = *Most or Almost All the Time*). Higher scores indicate greater depressive symptoms. The internal consistency of this questionnaire in our sample was very good ($\alpha = .90$).

To evaluate anxiety symptomatology, we used The Penn State Worry Questionnaire, which is a 16-item measure that uses a 5-point Likert scale (PSWQ; Meyer et al., 1990). The questionnaire measures worry and general anxiety disorder. The scale ranges from 1 to 5 for each question (1 = *Not at all typical of me*; 5 = *Very typical of me*). The total score is calculated by summing the first 11 items and the reverse-scores of the other 5 items. Higher PSWQ scores reflect greater levels of pathological worry. The internal consistency for our sample was also very good ($\alpha = .91$).

Child psychopathology. We used the MacArthur Health and Behavior Questionnaire (version 1.0), on which parents provided information about their children's functioning (HBQ; Essex et al., 2002). The HBQ has multiple scales (e.g., depression, externalizing symptoms,

conduct disorders, attention-deficit/hyperactivity disorder symptoms, etc.) For this study, we focused on the depression subscale only. Responses on the HBQ ranged from 0 to 2 (0 = *Never or not true*; 2 = *Often or very true*). The depression subscale is calculated as the mean of all the items on the subscales. Reliability for the subscale in our sample was adequate ($\alpha = .69$).

We used the Screen for *Child Anxiety Related Disorders* (SCARED; Birmaher et al., 1999) to assess anxiety. The SCARED is a 41-item inventory that uses a 3-point Likert scale (0 = *Not True or Hardly Ever True*; 2 = *Very True or Often True*) that screens for symptoms of anxiety disorders in children. We used the version of this questionnaire in which parents report on their child's symptoms. We focused on the general score that is calculated by summing up all items. A score higher than 25 on this scale may indicate the presence of an anxiety disorder. Reliability was excellent ($\alpha = .90$).

RESULTS

Gender Differences. There were no gender differences for any of our variables of interest (i.e., parent-child conflict, parent anxiety, parent depression, child anxiety, and child depression), at all t s < 1.888 , p s $> .061$.

Correlations. As expected, there was a positive significant correlation between parent anxiety and child anxiety, $r(170) = 0.324$, $p < .001$. Additionally, there was a positive significant correlation between parent depression and child depression, $r(173) = 0.412$, $p < .001$. A positive significant correlation between parent depression and child anxiety was also present, $r(174) = 0.319$, $p < .001$. There was also a significant positive correlation between parent anxiety and child depression, $r(170) = 0.299$, $p < .001$. Parents with psychopathology, either depression or anxiety, were linked to child psychopathology of either depression or anxiety. Therefore, parental anxiety was not specifically correlated to only child anxiety. These correlations show that the presence of parent psychopathology is correlated to their child having psychopathology even though the symptoms may not be the same as their parents. However, there were no significant associations between parent-child conflict and child anxiety, $r(172) = 0.015$, $p = .841$, or between parent-child conflict and child depression, $r(173) = -0.008$,

$p = .915$. Age was significantly correlated only with child depression, $r(183) = .191$, $p = .009$.

Regression model for child depression. Given the correlation of age with child depressive symptoms, at the first step of this model we entered children's age as a covariate. This step was significant $F(1, 164) = 5.308$, $p = .022$, $R^2 = .031$ and age was a significant covariate ($b = .023$, $t = 2.304$, $p = .022$). At the second step, we entered parents' depressive symptoms and parent-child conflict. This step was significant $F\Delta(2, 162) = 14.803$, $p < .001$, $R^2\Delta = .150$. As expected, parents' depressive symptoms predicted child depressive symptoms ($b = .010$, $t = 5.433$, $p < .001$), but parent-child conflict did not predict child depressive symptoms ($b = -.011$, $t = -.489$, $p = .626$). At the third step, we added the interaction between parents' symptoms and parent-child conflict, but this step of the model was not significant, $F\Delta(1, 161) = 14.803$, $p < .001$, $R^2\Delta = .013$, suggesting parent-child conflict did not directly relate to children's depressive symptoms, nor did it moderate the effect of parent depressive symptoms on child depressive symptoms.

Regression model for child anxiety. At the first step of this model, we entered parents' anxiety symptoms and parent-child conflict $F(2, 160) = 9.668$, $p < .001$, $R^2 = .108$. This first step was significant. As expected, parents' anxiety symptoms predicted child anxiety symptoms ($b = .231$, $t = 4.395$, $p < .001$), but parent-child conflict did not predict child anxiety symptoms ($b = -.212$, $t = -.289$, $p = .773$). At the second step, we added the interaction between parents' symptoms and parent-child conflict. This step was significant $F\Delta(1, 159) = 6.667$, $p = .011$, $R^2\Delta = .036$. The interaction of parents' anxiety symptoms and parent-child conflict was significant ($b = .131$, $t = 2.582$, $p = .011$). A closer look at the interaction (**Figure 1**) revealed that higher parent anxiety was associated with more child anxiety, but only for children who experienced high parent-child conflict ($b = .354$, $t = 4.669$, $p < .001$). For children who experienced low parent-child conflict during the task, parents' anxiety did not relate to children's anxiety ($b = .092$, $t = 1.157$, $p = .249$).

DISCUSSION

The current study was conducted to examine whether

conflict moderated the relation between parental psychopathology and child psychopathology. We hypothesized that parent depression and anxiety would be related to child depression and anxiety, respectively, and that parent-child conflict would moderate both associations. Specifically, we expected parent psychopathology to be a particularly important predictor of child psychopathology for children experiencing high parent-child conflict. As expected, both parents' anxiety and parents' depressive symptoms were related to children's anxiety and depressive symptoms. However, we found the expected moderating effect of parent-child conflict only for anxiety and not for depressive symptoms. Thus, the results only partly support our hypotheses.

The interaction pattern for anxiety showed that there was no relation between parent and child anxiety for low-conflict dyads, but a positive association between parents' and children's anxious symptoms among high-conflict dyads, as shown in *figure 1*. High parent-child conflict coupled with parent anxiety related to symptoms of anxiety in children, an additional risk factor that children who experienced low conflict did not have. Conflict may be particularly important for anxiety, because high levels of

stress within the dyad are likely linked with other aspects of difficult parenting, such as high levels of parental control, which has been found to increase the chance that particular types of anxiety symptoms and disorders will develop among children (Wood, 2006). For example, it has been shown that mothers were more involved and intrusive in a difficult and stressful situation (Hudson & Rapee, 2000). In the context of high conflict interactions, parents are likely engaging in behaviors that put too much pressure on the child, generating anxiety over the inadequacy to handle the stressful situation (Van Der Bruggen et al., 2008). In addition, we believe that high conflict situations with a parent may serve as an additional stressor to children, who will may already not feel capable to handle these types of situations because they are used to their parents taking control. This increased stress can be an additional possible mechanism through which child anxiety symptoms become worse through childhood.

We found the moderating effect for anxiety but not depression, perhaps because parents' anxiety symptoms are more likely to prompt parents to want to be in control of the situation and dominate their children more so than would parents' depressive symptoms. In turn, this greater

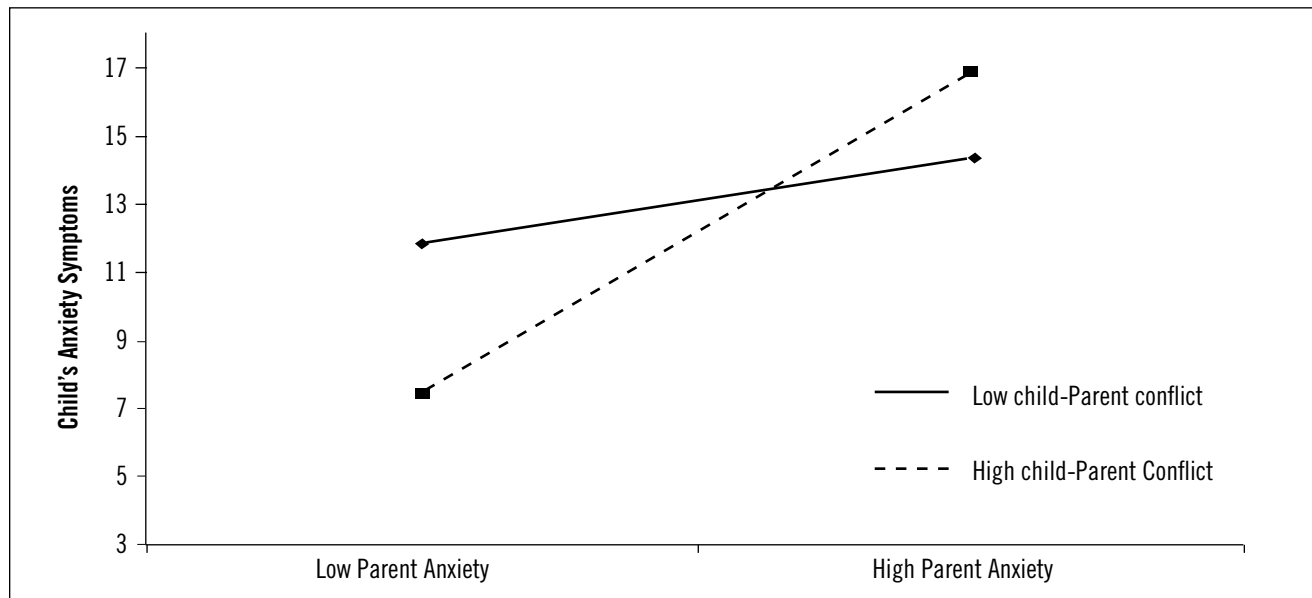


Figure 1. Interaction between parents' anxiety symptoms and parent-child conflict predicting child's anxiety symptoms.

Low child-parent conflict: $b = .092$, $t = 1.157$, $p = .249$

High child-parent conflict: $b = .354$, $t = 4.669$, $p < .001$

need to be in control of the child's behavior likely leads to high conflict within the dyad, and in turn, more anxiety symptoms for the child. Evidence supports the idea that parents' anxiety level influences parental control behaviors as a mechanism through which parents aim to avoid having their child encounter threatening situations (Van Der Bruggen et al., 2008). In this case, parents' anxiety is associated with them controlling the stressful situation, which can create conflict with their children as they put pressure on children to behave in certain ways. It is possible we also found this effect because of the behaviors associated with each of these types of psychopathological symptoms. For example, a depressed parent may not express interest in the activity with their child, resulting in fewer attempts at controlling the situation, and in turn, less conflict within the dyad. On the other hand, anxious parent may be overly interested and over involved in the activity, increasing the likelihood of conflict happening during the task.

Like any other study, there were limitations that should be acknowledged. For one, parents' and children's psychopathology symptoms were both provided by the parent, so there may have been some reporter bias. For example, anxious parents could have rated their child as being more anxious than they really are. In addition, the environment could have also played a role when it came to the conflict observation we used. Doing the task in a lab setting could have made parents or children more anxious and could have led to more frustration and conflict, or it could have led to parents interacting with their children in a more socially acceptable way. For one, parents could have acted more kindly when interacting with their children,

because they were aware that they were being watched. Hence, there was a possible chance of observer effect as subjects could have changed their behavior because they know they are being studied.

It would be valuable to do further research on other types of parent and child interactions that may additionally moderate symptoms of child psychopathology. For example, parents' socialization of emotional responses might also moderate the relation between parent and child symptoms. Also, it would be useful to study why parents with psychopathology display some behaviors more often than parents not experiencing symptoms of psychopathology. These behaviors can shed light on why these parents have different interactions and relationships with their children. Future studies should also aim to study parent-child interactions in more naturalistic settings to better assess their behaviors during an interaction. By doing the observation in the family's household instead of a lab, the participants may act the way they usually would, without pressure of having to act in a more socially desirable manner.

CONCLUSION

The current study expands our knowledge of the link between parent and child psychopathology by highlighting parent-child conflict as an important moderator of child anxiety but not depressive symptoms. This is important to note when attempting to reduce risk factors in children's lives that can lead to psychopathology and has clear implications for clinical work as clinicians must be aware of the myriad factors that play a role in child psychopathology.

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White Matter Integrity and Subclinical Depression: A Diffusion Tensor Imaging Study

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ABSTRACT

Major Depressive Disorder (MDD) is characterized by the persistent presence of at least five depressive symptoms over a two-week period. These symptoms must include either depressed mood, or loss of interest or pleasure. Early identification, and ultimately treatment, of depression may be accomplished by identifying neural markers of individuals at risk for MDD, including those with subclinical depressive symptoms. Neuroimaging studies have shown that MDD is associated with impairments to integrity in white matter tracts such as the corpus callosum and internal capsule. However, it is unclear whether these same structures also are disrupted in subclinical depression. The present study sought to examine this question through utilizing diffusion tensor imaging to assess white matter integrity as a function of Geriatric Depression Scale Short Form (GDS-SF) scores. Using a median split of GDS-SF scores, statistical analyses revealed no difference in white matter integrity between low risk and high risk depression groups. However, there was a nonsignificant trend ($p=0.072$) such that higher GDS-SF scores were associated with decreased white matter integrity localized to the corpus callosum, right internal capsule, left cingulum, external capsule and fornix. This finding extends previous research on MDD by providing evidence for similar neural correlates of subclinical depression. This may provide insight into the development of MDD and ultimately aid diagnostic and treatment efforts with early identification and intervention.

Keywords: Major depressive disorder, diffusion tensor imaging, white matter integrity



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Nicole Huffman is a fourth year neuroscience major who has studied white matter integrity in subclinical depression for almost two years under the advisement of Dr. Ilana Bennett. Nicole is a member of University Honors, and an editor for the Undergraduate Research Journal.

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INTRODUCTION

Major Depressive Disorder (MDD) is characterized by sad, empty, or irritable mood, and is accompanied by somatic and cognitive alterations that significantly affect an individual's ability to function (American Psychiatric Association, 2013). Diagnosis is made according to the persistent presence of at least five depressive symptoms over a two-week period. These symptoms must include either depressed mood, or loss of interest or pleasure. Other possible depressive symptoms are: significant changes to appetite, weight loss or sleeping patterns; fatigue or loss of energy; feelings of worthlessness and inappropriate guilt; diminished ability to think or concentrate; and recurring thoughts of death or suicide (American Psychiatric Association, 2013; Reynolds and Kamphaus, 2013). As of 2010, MDD was the second leading cause of years lived with disability, the leading cause of burden, and a contributor to the burden allocated to suicide and ischemic heart disease (Ferrari, Charlson, Norman, Patten, Freedman, Murray, Vos and Whiteford, 2013). An estimated 16.1 million adults over the age of 18 - representing 6.7% of the US population - had at least one major depressive episode in 2016 (Substance Abuse and Mental Health, 2017). Given this extensive impact of MDD on individuals and society, it is increasingly important to identify, and ultimately treat, depression early.

This goal may be addressed through identifying subclinical depression, which has been shown to increase an individual's risk of developing MDD (Wesselhoeft, Heiervang, Kragh-Sorensen, Sorensen and Bilenberg, 2016). Subclinical depression is characterized by the presence of clinically relevant depressive symptoms without meeting the criteria for MDD (Cuijpers, Koole, van Dijke, Roca, Li and Reynolds, 2014). It is highly prevalent, has its own serious consequences for an individual's quality of life, and is associated with increased economic costs and mortality rates (Cuijpers and Smit, 2008; Cuijpers, Koole, van Dijke, Roca, Li and Reynolds, 2014).

One method for identifying subclinical depression is the Geriatric Depression Scale Short Form (GDS-SF). This 15-item self-reported questionnaire evaluates the presence or absence of various depressive symptoms, such as a lack of energy and the inability to feel pleasure (Sheikh

& Yesavage 1986). The GDS-SF has been demonstrated as a valid and reliable assessment in both young and old adults (Ferraro & Chelminski 1996; Sheikh & Yesavage 1986). Scores in the 0 to 4 point range indicate limited to no presence of depression, while scores in the 5 to 10 point range indicate subclinical depression, and scores from 11 to 15 indicate major depression (Cuijpers and Smit, 2008).

Another method with promise for identifying depression in the subclinical range is neuroimaging. For example, diffusion tensor imaging (DTI) is a structural magnetic resonance imaging (MRI) technique that measures the diffusion of water molecules in the brain. The rate and orientation of diffusion is constrained by the cellular environment and thus is thought to reflect the integrity of underlying white matter. That is, water will diffuse in all directions (isotropic diffusion) when there are no restrictive structures present, as seen in the ventricles, which only contain cerebrospinal fluid. In contrast, water diffusion is restricted (anisotropic diffusion) in the presence of neurons, diffusing faster along the myelinated axons of white matter tracts relative to the less coherent diffusion around cell bodies in grey matter.

DTI measures of diffusion are used to calculate multiple integrity metrics, including fractional anisotropy (FA) and mean diffusivity (MD). FA describes the degree of anisotropic diffusion, and is sensitive to fiber orientation. An FA value approaching one indicates that diffusion is anisotropic, or restricted, and suggests intact white matter integrity. MD measures the average rate of diffusion. Lower MD values indicate increased anisotropy, and suggest intact white matter integrity (Soares, et al., 2013; Alexander, Lee, Lazar & Field, 2007).

Recent DTI studies have shown that MDD is associated with decreased integrity in white matter throughout multiple brain regions, but especially in the corpus callosum and internal capsule, seen in *Figure 1* (see Chen, et al, 2016 for review). These studies primarily compared white matter integrity in individuals diagnosed with MDD to normal controls using tract-based spatial statistics (TBSS), in which integrity measures (FA, MD) are compared within a "skeleton" of white matter voxels, or volume elements, common to all participants. However, it remains to be

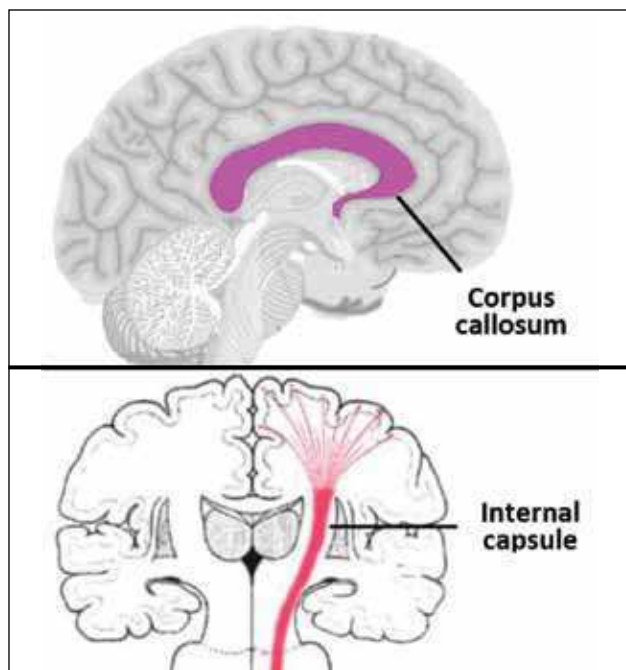


Figure 1 - Regions of Interest
Diagrams showing the anatomical location of the corpus callosum (top, in purple) and internal capsule (bottom, in red).

examined whether white matter tracts that are disrupted in MDD also are disrupted in subclinical depression.

To test the hypothesis that subclinical depression is associated with white matter integrity declines similar to those seen in MDD, the present study used TBSS to assess white matter integrity as a function of GDS-SF scores. Subclinical depression was assessed via group comparison between low depression risk and high depression risk groups based on a median split of the GDS-SF scores. Further analyses examined relationships between white matter integrity and GDS-SF scores within the FA and MD skeleton. Overall, it was predicted that higher GDS-SF scores would relate to worse integrity (lower FA, higher MD), particularly in the corpus callosum and internal capsule. Such a finding would support the notion that the white matter tracts impaired in MDD are also impaired in subclinical depression.

2. METHODS

2.1 Participants

A sample of 40 young adults (24 males) aged 18-24 years were recruited via the Psychology Department Research Pool at the University of California, Riverside. Participants

were compensated with class credit. All procedures were approved by the University of California, Riverside Institutional Review Board.

Prior to participation, all individuals were screened for neurological disorders (stroke, epilepsy, brain tumor, etc.), psychiatric disorders (bipolar disorder, schizophrenia, etc.), other medical conditions (alcoholism, HIV, diabetes, etc.), and contraindications to MRI scanning (non-removable ferrous materials, current pregnancy, claustrophobia, and head or neck tattoos).

2.2 Depression measure

Participants completed a series of neuropsychological assessments that included the GDS-SF. The GDS-SF was scored as a total number of points out of a possible 15.

2.3 Diffusion Tensor Imaging

2.3.1 Acquisition

Diffusion images were obtained using a 3T Siemens Prisma MRI fitted with a 32-channel head coil. Fitted padding was used to minimize head movements. Two diffusion-weighted echo-planar imaging sequence were acquired using separate anterior to posterior and posterior to anterior acquisition sequences with the following parameters: time repetition (TR)/time echo (TE) = 3500/102 ms, field of view (FOV) = 218×218 mm, 72 axial slices, and 1.7 mm³ spatial resolution. For each sequence, gradients ($b = 1500$ and 3000 s/mm²) were applied in 64 orthogonal directions, with six images having no diffusion weighting ($b=0$).

2.3.2 Preprocessing and Quality Control

Diffusion-weighted data were separately processed for each participant using a combination of FSL (FMRIB Software Library; Behrens et al. 2003) and AFNI (Analysis of Functional NeuroImages; Cox 1996). To correct for head movement and eddy currents, all volumes were aligned to the $b=0$ image (eddycorrect). Diffusion tensors were independently fit to each voxel (DTIfit) using a binary mask to limit tensor fitting to brain space (3dSkullStrip). The output yielded voxel-wise diffusion images of FA and MD.

Before proceeding to analysis, each participant's data was manually assessed for quality. This process involved checking for precise image alignment, minimal head

motion, accurate brain extraction, good tensor fitting, and minimal voxels containing the theoretically impossible value of FA greater than 1. Quality control screening resulted in the exclusion of 3 participants.

2.3.3 Analysis

Tract-Based Spatial Statistics (TBSS) was utilized to perform exploratory skeleton-wise analyses (i.e., voxel-wise analyses within the mean skeleton) separately for each diffusion index (FA and MD) (Smith et al. 2006). The FA map of each participant was aligned nonlinearly to the FMRIB58_FA_1-mm template in MNI152 standard space. These aligned images were then averaged across all individuals to generate a mean FA image, which was used to produce a “skeleton” of white matter voxels common to all participants. Non-white matter voxels were excluded by thresholding FA at 0.2. The same nonlinear registrations used for FA images were then applied to each participant’s MD image.

In order to enhance cluster-like structures without prior definition of a cluster forming threshold, threshold-free cluster enhancement (TFCE) was employed (Smith and Nichols, 2009), yielding statistical maps that were FWE-corrected for multiple comparisons across space (TFCE, $P < 0.05$). Skeletonized data for both diffusion indices (FA, MD) were subjected to independent t-tests between low depression risk and high depression risk groups as determined by a median split at a value of 3, and to skeleton-wise correlations with GDS-SF scores.

3. RESULTS

3.1 Behavioral Data

GDS-SF scores ranged from 0 to 9 with a median of 3, which was used to split participants into separate low ($n = 21$, $M = 1.4$, $SD = 1.2$) and high ($n = 19$, $M = 5.3$, $SD = 1.5$) depression risk groups. An F-test revealed equal variances between groups, therefore an independent t-test assuming equal variances was conducted and revealed a statistically significant difference in GSD-SF scores between the two groups ($p < 0.01$). These behavioral results, along with the frequency of each score, are summarized in **Figure 2**.

3.2 Imaging Data

Group differences in white matter integrity were assessed using separate skeleton-wise t-tests between low risk

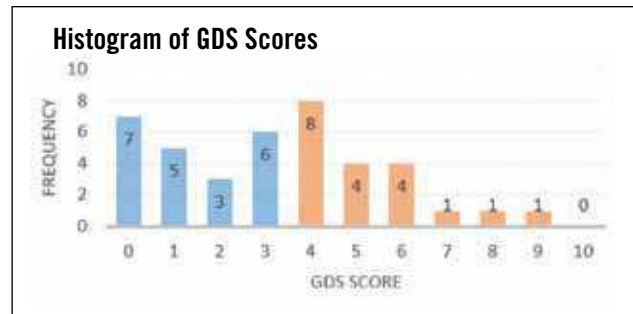


Figure 2 - GDS Behavioral Data

The above histogram shows the frequency of each score. A median split at a score of three resulted in a low risk depression group (blue; $N=21$; $M=1.4$) and a high risk depression group (orange; $N=19$; $M=5.3$). An independent t-test between the two groups showed a statistically significant difference between the means ($p = 5.98 \times 10^{-11}$).

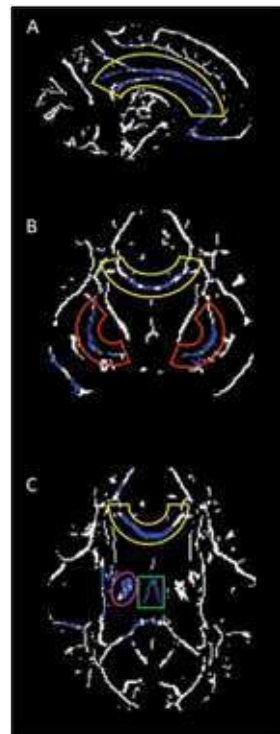


Figure 3: Regions Exhibiting a Positive Correlation between MD and GDS

Blue areas show the nonsignificant positive trend between MD and GDS ($p=0.072$) within various white matter tracts of the brain, as noted below.

A) Right cingulum and corpus callosum (both in yellow)

B) Corpus callosum (in yellow) and external capsule (in red)

C) Corpus callosum (in yellow), fornix (in green) and the right internal capsul (in pink).

and high risk depression groups for both FA and MD. No results approached significance ($p > 0.05$). The same pattern of null results was found when further analyses split participants at a GDS-SF score of 2 based on the bimodal distribution, and at a score of 5 based on the subclinical threshold reported in the literature.

To further assess whether white matter integrity is sensitive to subclinical depression symptoms, skeleton-wise correlations were conducted between GDS-SF scores

and each diffusion index. A nonsignificant positive trend ($p = 0.072$) was observed between MD and GDS-SF, such that the risk of depression increased as white matter integrity decreased. This effect was localized to the corpus callosum, internal capsule, external capsule, left cingulum, and fornix, as shown in *Figure 3*.

No areas showed a negative correlation between MD and GDS-SF, and neither the positive or negative correlation between GDS-SF and FA approached significance ($p > 0.16$).

4. DISCUSSION

White matter tracts implicated in MDD have been extensively examined (Chen, et al, 2016; Kieseppa, et al, 2010; Liao, et al, 2013; Xiao, He, McWhinne & Yao, 2015). The present study sought to extend this work by examining whether similar impairments in white matter integrity are seen in subclinical depression. Results revealed three main findings. First, in partial support of our hypothesis, there was a nonsignificant positive trend between GDS-SF and MD. Second, there was no difference in white matter integrity between low and high depression risk groups. Third, the relationship between GDS-SF and FA was not significant.

Consistent with our hypothesis that similar declines in white matter integrity are seen in MDD and subclinical depression, we observed a non-significant trend between GDS-SF and MD in the corpus callosum, right internal capsule, external capsule, left cingulum, and fornix. This finding parallels, and expands upon, previous research in MDD which found significant decreases in white matter integrity in the corpus callosum and internal capsule of depressed patients relative to healthy controls (Chen, et al, 2016). Importantly, degradation of white matter in the aforementioned regions may be associated with particular symptoms of MDD. For example, the internal capsule, which connects the thalamus to prefrontal cortex, plays a role in executive functions such as the planning of complex behaviors. The corpus callosum is the largest commissure, connecting the cerebral lobes; and integrates motor, sensory and cognitive functions. Disruptions to these areas may account for previous reports of impaired cognitive control in MDD (Hertel, 1997). Similarly, the left cingulum connects the left cingulate gyrus to the entorhinal cortex, and is involved in the appraisal of pain and the reinforcement of behavior that reduces it. This

may account for previous reports of comorbidity between pain and depression (Goesling, Clauw and Hasset, 2913). Finally, the fornix connects the hippocampus to the hypothalamus, septal nuclei and nucleus accumbens; and is involved in limbic functions and recall memory. This may account for the disturbances in affect and memory, which are assessed by the questions on the GDS-SF (i.e. Do you feel happy most of the time? Do you have more problems with memory than most?).

The observation that there were no group differences in white matter integrity between low and high depression risk groups was consistent across all three grouping methods - median split, bimodal split, and threshold split. This pattern of null results is at odds with previous reports of significant differences in white matter integrity in MDD patients (see Liao, et al, 2013 for review). This contradictory finding could be explained by two interpretations. First, collapsing subjects into high and low risk groups may obscure the individual differences in white matter integrity that are related to subclinical depression. Alternatively, the pathophysiology of subclinical depression may not be developed enough to be detectable in a group comparisons fashion. In partial support of these interpretations, our correlation analyses revealed a nonsignificant trend between white matter integrity (MD) and the presence of depressive symptoms (GDS-SF score).

In contrast, the positive correlation between GDS-SF and FA did not reveal the expected trend. This contradictory data may be explained by the fact that FA seems to be more sensitive to the organization and directionality of tissues, while MD tends to be more sensitive to non-directional measures such as the degree of myelination and the number of axons. Based on this interpretation, the present data may indicate that impaired white matter integrity in subclinical depression is a result of myelination or axon number, as opposed to fiber directionality and organization. However, there is still much more to be elucidated, as the characteristics of integrity measured by FA and MD overlap to a great extent.

The current study analyzed white matter integrity as a function of GDS-SF scores to examine subclinical depression in young adults. Findings indicate that there is no significant differences between low risk and high

risk depression groups; but that increased MD, and therefore impaired white matter integrity, shows a strong nonsignificant trend with subclinical depression. Notable limitations include sample size and GDS-SF score distribution. The relatively small number of subjects may have limited the power of the present analysis, while the number of participants who truly scored in the subclinical depression range was moderately limited. Future studies could overcome these limitations by recruiting a larger

number of participants, with a higher percentage scoring in the subclinical depression range. Regardless of these limitations, the present study suggests that a potential relationship between subclinical depression and impaired white matter integrity in the same tracts implicated in MDD may exist. Such findings could provide insight into the development of MDD and ultimately aid diagnostic and treatment efforts with early identification and intervention.

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Does Parenting Moderate the Relation between Stress and Children's Emotion Regulation?

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¹ Department of Psychology

ABSTRACT

Parenting behaviors greatly influence children's ability to regulate their emotions and handle stressful situations. Stressful life events can be particularly problematic for children as they are less able to effectively manage these situations. Parenting behaviors that are warm and focus on directly helping the child handle negative emotions may serve as protective factors against the negative effects of stress on children's regulatory abilities. The aim of this study was to explore the role of parental warmth and parents' emotion-focused reactions as moderators of the effect of stress on children's emotion regulation. A total of 184 children between the ages of three to eleven years old ($M = 7.66$, $SD = 2.30$) participated in this study. Parents reported on their child's emotion regulation, exposure to stressful life events, and on how they, as parents, deal with their child's negative emotions. Parental warmth was coded from an interactive task. Results showed that parental warmth moderated the relation between stress and child emotion regulation, such that children of highly warm parents had better emotion regulation even when experiencing high stress. Parents' reactions to their child's negative emotions moderated the effects of parental warmth on child emotion regulation, such that parental warmth was particularly important for children of parents who place less focus on their child's emotions when they experience a negative event. Our findings partially supported our hypotheses and offer new insight into the importance of parental warmth as a protective factor against the negative consequences of stress on children's emotional functioning.

Keywords: Parental warmth, emotion regulation, emotion-focused reactions, stress, parenting, parent-child interactions



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Karina Lopez-Villegas is a third-year Psychology major with a passion for child development. With the support of her graduate student, Laura E. Quiñones-Camacho, and faculty advisor, Dr. Davis, she has been studying for the past year how stress and parenting behaviors influence child emotion regulation throughout the childhood years. Using her newly acquired knowledge she plans to pursue her PsyD in Clinical Psychology to become a Child Psychologist specializing in counseling hospital bound children in hopes of preventing post-traumatic disorders.



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Dr. Elizabeth Davis is an Assistant Professor in the Psychology Department at UC Riverside. She earned her PhD in Developmental Psychology from the University of California, Irvine in 2009. Research in the Emotion Regulation Lab focuses on understanding how emotion regulation relates to adaptive outcomes (e.g., learning) and maladaptive outcomes (e.g., anxiety) in childhood. Emotion regulation can be broadly defined as the set of processes by which people influence the timing, expression, and experience of their emotions. The lab's work to date has aimed 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 whether they can regulate emotion effectively. This research also focuses on identifying mechanisms responsible for effective emotion regulation (e.g., attentional focus) to explain why certain emotion regulation strategies attenuate negative emotion and distress. Ultimately, this program of research can be viewed as providing an empirical basis for interventions aimed at improving children's emotion regulation abilities and mitigating risk for maladaptive outcomes.

INTRODUCTION

Many studies have shown that children are greatly influenced by their environment (Masten, 1998). Parental characteristics and parenting behaviors are particularly important influences during childhood (Hughes, 2014). Children learn from their parents through coaching and mimicking of socially acceptable behaviors. Throughout childhood, these parenting practices are internalized by the child, shaping their future behavior. The role of parents' behaviors is particularly important for emotional expression and regulation, as children learn how to manage themselves in different emotional situations in large part through observing and interacting with their parents (Moges & Weber, 2014). Although there is substantial evidence for the role parents play in shaping a child's ability to regulate their emotions (Murphy, 2014), less is known about how these patterns might be different for children experiencing high stress (e.g., children who have experienced violence, illness, or death of a loved one).

Experiencing high stress early in life has been associated with many maladaptive outcomes (Dye, 2018), thus, understanding potential risk and protective factors in the face of high stress is necessary to better help children facing these challenges. The experience of high stress might be particularly difficult for children as they are still developing their ability to manage negative and stressful situations. Emotion regulation is defined as the ability to modify emotional responses in the service of a goal (Gross, 2015). For example, in an event of a scary movie, one can change what is on one's mind by thinking that the creature is not real and that it is just a projected image, thus reducing scared feelings. Throughout childhood, children are learning how to change their emotional responses and their goals to change how they feel about negative events (Davis, Levine, Lench, & Quas, 2010). Children experiencing high stress might feel hopeless or defenseless in the situation as they might feel they have no control over the situation and are unable to manage or regulate the negative emotions caused by the stressors. Successfully being able to change how they feel about stressful events can allow them the chance to work through the negative emotions, resulting in more adaptive functioning (Davis et al., 2010).

Parents' contributions to their children's developing

emotion regulation abilities have an impact on how well children will be able to change how they feel when they experience negative emotions. Mothers seem to be particularly important for helping socialize adaptive emotion regulation (Morris, 2007; Morris, Morris, Silk, & Steinberg, 2011). More work is needed to better understand how parents' (especially mothers') socialization of emotion regulation might differ within families living in more versus less stressful environments. This study aimed to shed light on this important gap in our knowledge by exploring two different aspects of parental emotion socialization: parental warmth and parental emotion-focused reactions to their child's negative emotions. Parental warmth refers to verbal and physical actions that provide children with supportive attributes while parental emotion-focused reactions refers to parents' verbal responses to avoid or decrease negative emotions that their children may be feeling.

Current Study

In this study, we examined parental responses to children's negative emotions and the warmth of parents' responses during a stressful laboratory task to assess the different role of these two aspects of parental emotion socialization on children's developing emotion regulation. Moreover, we explored the moderating role of these two aspects of parental emotion socialization on the relation between stress and emotion regulation. We hypothesized that parents who focused more on their child's emotions and were warmer toward them during a stressful task would have children with better emotion regulation even in the presence of high levels of environmental stress.

METHOD

Participants

A total of 184 three to eleven year-old children ($M_{age} = 7.66$, $SD = 2.30$; 49.7% girls) took part in the study. This was a highly diverse sample, with children endorsed by parents as being Caucasian/White (18.2%), African American (10.7%), Hispanic (29.4%), Asian American (2.1%) and other ethnicities or more than one ethnicity (37.4%). Mothers' formal schooling ranged from grade school (1.6%) to a Doctoral degree (2.7%), with the majority-of the mothers reporting some college education (30.5%). Fathers' formal schooling ranged from Grade School (1.6%) to a Doctoral degree (3.6%) with the majority of

fathers having finished high school (31.6%). Household income ranged from \$15,000 or less (15.5%) to above \$100,000 (11.8%) with most families endorsing an income between \$21,000 to \$50,000 (32.1%).

Procedure

Families came to the Emotion Regulation lab at the University of California Riverside for a single visit. Before the study started, a trained research assistant obtained informed consent from the parents and assent from the children. Parents reported on their child's sources of stress, their own reactions to their child's negative emotions, and their child's emotion regulation with surveys while children completed a series of tasks (not considered here). After each task, children were given the opportunity to take a short break, enabling them to return to a calm and non-emotional state between tasks and minimizing any emotional carryover from previous tasks. For the second half of the study, parents joined their child for some dyadic tasks, of importance for this study is a frustrating Lego task in which we measured parental warmth (described below). At the end of the study, families received a small honorarium and the child took home a toy. The procedures were always completed in English.

Stimuli and Measures

Child stressful life events. Parents were asked to indicate if their child had experienced a series of stressful life events in the past year using the *Children's Stressful Life Events Questionnaire* (CSLEQ; Sandler & Ramsay, 1980). There were 32 stressors that parents could potentially endorse. To assess a child's level of life stress, we summed all the stressors that the parents endorsed for their child. A higher number indicates the experience of more life stressors.

Parental warmth. During the second part of the study, parents joined their child to take part in some tasks, including a Lego-building task. This Lego task took a total of 10 minutes and was divided into two phases. In phase one, the parent and child were told they had five minutes to complete a Lego structure, however, the Lego structure was too complex to be completed during that time. During this phase, parents were asked to refrain from physically helping their child but were given the instruction manual for assembling the Lego structure. After this first five

minutes, the experimenter came in and told the parent and child that the parent was now allowed to physically help with building the Lego structure and that they would get five more minutes to work on the Lego structure. After giving the new instructions, the experimenter left the room. The departure of the experimenter indicated the start of phase two. In this phase, parent and child had another five minutes to work on the Lego structure, but this time parents could physically aid the child if they wished to do so. Parental warmth during this task was measured using a 5-point scale (1 = *low warmth*; 5 = *high warmth*) that took parents' behaviors and verbalizations into account for the entire Lego task (i.e., one global warmth code was applied for each family). For example, a parent who ignored the child even if they seemed distressed was scored as being low on warmth. On the other hand, a parent who was physically affectionate with their child during the task and gave frequent praise was scored as being high on warmth.

Parental reactions to children's negative emotions. To measure parents' emotion-focused reactions to children's negative emotions, we administered the *Coping with Children's Negative Emotions Scale* (CCNES; Fabes, Eisenberg, & Bernzweig, 1990). This scale consists of 12 vignettes describing a child's negative reaction to a hypothetical event and seven possible reactions that parents could have for each of the events. Parents reported how likely they were to react in each of the seven hypothetical events on a 7-point scale (1 = *very unlikely*; 7 = *very likely*). This scale yields six subscales that reflect specific types of reactions to children's expressions of negative emotion (*Distress reactions*, *Punitive reactions*, *Expressive encouragement*, *Emotion-focused reactions*, *Problem-focused reactions* and *Minimization reactions*). For this study, we focused only on the emotion-focused reactions subscale. Internal consistency was good ($\alpha = .84$). Higher scores indicate greater parental endorsement of the type of reaction.

Child emotion regulation. We used the *Emotion Regulation Checklist* to assess emotion regulation (ERC; Shields & Cicchetti, 1997). This scale consists of 24 items that form two subscales, an emotion regulation subscale, and an emotional reactivity subscale. We focused on the emotion regulation subscale. The emotion regulation

subscale consists of 12 items (e.g., “Can modulate excitement in emotionally arousing situations”) and the score for the subscale is calculated as the mean of all the items. Parents responded on a 4-point scale how much of the time their child was like the child described in each statement (1 = *never*; 4 = *always*). After removing one item from this scale that negatively impacted the internal consistency, the reliability of the modified scale in our sample was modest but acceptable ($\alpha = .67$). Higher scores indicate better emotion regulation.

RESULTS

Gender differences. There were no significant gender differences in any variables of interest, all t s < 1.135, p s > .258.

Correlations. There was a positive correlation between parental warmth and child emotion regulation, $r = .257$, $p = .001$, $n = 162$, as well as between parental emotion-focused reactions and child emotion regulation, $r = .274$, $p < .001$, $n = 176$. Contrary to our expectations, a high level of stress was not correlated with child emotion regulation, $r = -.093$, $p = .220$, $n = 176$. Additionally, parental emotion-focused reactions and parental warmth were not significantly correlated, $r = -.045$, $p = .569$, $n = 160$.

Regression model predicting child’s emotion regulation.

At the first step, we entered children’s level of stress, parents’ emotion-focused reactions, and parental warmth. This step was significant $F(3, 155) = 8.910$, $p < .001$, $R^2 = .147$. As expected, both parents’ emotion-focused reactions ($b = .124$, $t = 3.482$, $p = .001$) and parental

warmth ($b = .085$, $t = 3.663$, $p < .001$) predicted child emotion regulation, but level of stress did not ($b = -.018$, $t = -1.370$, $p = .173$). At the second step, we added the interactions between stress and parents’ emotion-focused reactions, stress and parental warmth, and parents’ emotion focused-reactions and parental warmth. This step was also significant $F_{\Delta}(3, 152) = 4.231$, $p = .007$, $R^2_{\Delta} = .066$. The interaction between parents’ emotion focused-reactions and parental warmth was significant ($b = -.070$, $t = -2.395$, $p = .018$), as was the interaction of stress and parental warmth ($b = -.019$, $t = -2.190$, $p = .030$). Interactions between these continuous variables were plotted at ± 1 SD (corresponding to low and high levels) from the mean (Aiken, West, & Reno, 1991) as is typical in developmental work. At the third and last step, we entered the three-way interaction of stress, parents’ emotion-focused reactions, and parental warmth, but this step was not a significant improvement to the model $F_{\Delta}(1, 151) = .676$, $p = .412$, $R^2_{\Delta} = .004$.

A look at the two-way interaction between parents’ emotion-focused reactions and parental warmth (see **Figure 1**) revealed that the level of warmth of the parent predicted children’s emotion regulation only for children who had parents who less often endorsed emotion-focused reactions to their child’s negative emotions (low emotion-focused reactions: $b = .170$, $t = 3.432$, $p = .001$; high emotion-focused reactions: $b = .036$, $t = .916$, $p = .361$). Therefore, a closer look at the second two-way interaction of stress and warmth (see **Figure 2**) revealed that children with low-warmth parents had overall lower emotion regulation

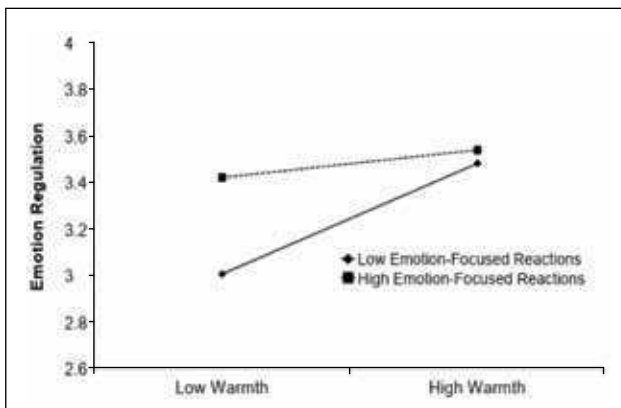


Figure 1. Two-way interaction of warmth and emotion-focused reactions predicting child’s emotion regulation. Low emotion-focused reactions: $b = .170$, $t = 3.432$, $p = .001$; High emotion-focused reactions: $b = .036$, $t = .916$, $p = .361$

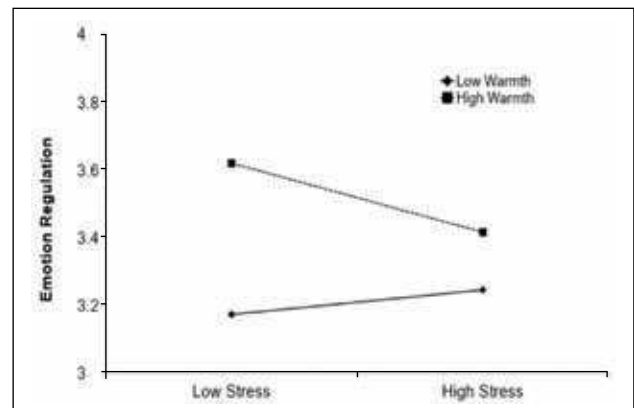


Figure 2. Two-way interaction of stress and warmth predicting child’s emotion regulation. Low warmth: $b = .006$, $t = .343$, $p = .732$; High warmth: $b = -.032$, $t = -2.257$, $p = .025$.

independent of the amount of stress they were experiencing ($b = .006, t = .343, p = .732$). On the other hand, children with high-warmth parents showed the expected association of higher stress being associated with poorer emotion regulation ability ($b = -.032, t = -2.257, p = .025$).

DISCUSSION

The goal of our study was to examine parental warmth and parental emotion-focused reactions as protective factors against the deleterious effect of stress on children's emotion regulation. We hypothesized that children whose parents primarily focused on their child's emotions during a negative event to make them feel better and who were warmer towards their child during a stressful laboratory task would show a greater ability to regulate their emotions. Our findings partially supported our hypothesis that parental emotion socialization would buffer against the effects of stress, but this was true only for parental warmth (not for emotion-focused reactions). Additionally, we found an unexpected moderating effect, that emotion-focused reactions qualified the link between parental warmth and child emotion regulation.

It is not surprising that parental emotion-focused reactions were associated with child emotion regulation. Greater parental focus on how to manage emotions would support children's development by giving them more opportunity to practice their emotion regulation skills, supported by emotion regulation modeling and coaching from parents. It is interesting to note that parental emotion-focused reactions interacted with parental warmth, such that parents high in emotion-focused reactions had children with better emotion regulation independent of their level of warmth. One reason why emotion-focused reactions might have moderated the effects of parental warmth on children's emotion regulation could be that in the absence of direct guidance on how to manage negative emotions. Having a parent who was still warm, and comforting might have given children the confidence and support to manage difficult experiences better. Our study adds to the growing body of research on how parenting can influence child emotion regulation and advances research in this area by

showing how parental warmth and behavior focused on children's emotions are both important for understanding children's developing emotion regulation abilities.

The main aim of our study was to investigate the protective effect of these parenting practices against the negative effects of stress on emotion regulation. Our findings suggest that parental warmth is a particularly important aspect of parental emotion socialization for children's emotion regulation in the presence of stress. Specifically, children with warmer parents had better emotion regulation even in the presence of high stress. A parent's ability to connect with their child in the presence of uncontrollable stress and continue to be warm and supportive towards them could give children a sense of security and the confidence needed to regulate their emotions on their own.

Some limitations of the current study should be noted. First, parents were the ones who reported on their child's stressors and their child's emotion regulation. This was done because of the wide age range in this study, but it may have biased our results as most measures relied on parent report. Future studies should have children report on their own experiences and reactions through interviews with an experimenter to capture the child's subjective experience and perception of the stressors they encounter. This would provide another perspective on children's experiences of stress that will ultimately help clarify the role of parental emotion socialization on children's emotion regulation across various developmental contexts. Additionally, our study was cross-sectional, which limits what can be said about how the pattern of associations we detected may change over time. The directionality of effects and potential causal explanations for the results. Therefore, future studies should adopt a prospective longitudinal approach to assess how these associations change throughout children's lives.

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The Neurotoxic Effects of Cycads and Metals: A Review

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ABSTRACT

The bioaccumulation of environmental toxins as possible risk factors in the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PDC) is studied in three foci of the Western Pacific: Guam, the Kii Peninsula, and West Papua New Guinea. The objective of this study was to evaluate the best evidence on the exogenous causes of ALS/PDC, with emphasis on the role of cycads, iron, and manganese in the Western Pacific foci, by performing a systematic review of major electronic databases using predefined criteria, 68 of which met the selection criteria. Two major environmental hypotheses are associated with this enigmatic disease: the vegetal hypothesis, which focuses on the neurotoxic and genotoxic properties of the cycad, and the mineral hypothesis, which focuses on the neurotoxic properties of metals. Although typically studied independently, environmental data suggests these two hypotheses may, in fact, converge. Epidemiologic research investigating the association between exposure to environmental toxins and ALS/PDC has proven inconclusive. Nevertheless, possible causal links indicate a need for more holistic research to not only better understand ALS/PDC, but also glean new insights regarding the associated neurodegenerative diseases.

Keyterms: amyotrophic lateral sclerosis and parkinsonism-dementia complex; Alzheimer's disease; Parkinson's disease; Western Pacific; cycad; iron; manganese; neurotoxicity



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Dr. Hu obtained his Ph.D. in medical physics from the University of Chicago in 1988. From 1990-2002, he was on the faculty of the University of Minnesota, where he became a full professor in 1998. From 2002-2016, he was Professor and Georgia Research Alliance Eminent Scholar in Imaging in the Wallace H. Coulter joint department of biomedical engineering at Georgia Tech and Emory University. In July 2016, Dr. Hu moved to UC Riverside to become professor and chair of bioengineering and director of center advanced neuroimaging. Dr. Hu has worked on the development and biomedical application of magnetic resonance imaging for 4 decades. As one of the early players, Dr. Hu has conducted extensive and pioneering work in functional MRI (fMRI).



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Brendan's passions is focused on researching the etiology of neurodegenerative diseases. In the future, he plans to pursue a PhD in the field of neuroscience.

INTRODUCTION

Amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS/PDC) is a prototypical, long latency neurodegenerative disease that, during the first decade after World War II, was reported to develop in extraordinarily high frequencies among three geographically and genetically distinct populations in the Western Pacific: the indigenous Chamorro residents of Guam,¹ the Japanese of Honshu Island's Kii peninsula in Japan,² and the West Papuan New Guineas of Irian Jaya, Indonesia.³ The clinical and neuropathological features of this disease are best studied among the Chamorro people of Guam and the Japanese living in the Kii Peninsula of Honshu Island. Least studied, and lacking neuropathological confirmation, is the Auyu and Jakai (Jaqai) linguistic groups in the southern lowlands of West Papua, the Indonesian side within New Guinea. This review explicates the polemical role of plants and minerals in the pathogenesis of ALS/PDC in the three foci of the Western Pacific.

This enigmatic and invariably fatal disease of the Western Pacific is characteristic of classical ALS, Parkinsonism, and Dementia. Although the insidious progression of neurodegenerative diseases is typically due to senescence, the age of onset for ALS/PDC can be as early as adolescence for the ALS phenotype and middle adulthood for the parkinsonian and dementia phenotypes.⁴ Those afflicted with this disease experience debilitating symptoms such as cognitive deficits, spasticity, and muscle atrophy leading to a vegetative state and death. Despite the dramatic decline of ALS/PDC incidence, the Western Pacific foci can be a valuable case-study for understanding the etiology of the associated neurodegenerative diseases.

The hallmark biomarker of ALS/PDC is polyproteinopathy, in which multiple proteins aggregate in the brain. In ALS/PDC, the affected brain accumulates a constellation of abnormal intracellular deposits (synuclein, β -amyloid, and transactive response (TAR)-DNA-binding protein 43 (TDP-43))⁵ but is dominated by telencephalic (anterior region of the forebrain) neurofibrillary tangles (NFTs), contributing to stark cortical neuron loss.⁶ Even though tauopathy, aggregation of tau protein in the brain, is a key characteristic of ALS/PDC, it cannot be distinguished from other neurodegenerative disorders; ALS/PDC has a great

degree of heterogeneity. The following examples elucidate this point: (1) the presence of hyperphosphorylated tau⁷ and α -synuclein negative inclusions found in ALS/PDC are also seen in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U), a neuropathological subtype of frontotemporal dementias;⁸ (2) tau isoform distribution, commonly associated with ALS/PDC, is observed in cases of Alzheimer's disease (AD);⁹ (3) cortical laminar distribution is linked to progressive supranuclear palsy (PSP);⁹ (4) possible malfunctioning of TDP-43 proteinopathy is seen in FTLD-U and ALS;⁹ and (5) leucine-rich-repeat-kinase 2 (Lrrk2), a gene that, when mutated, is seen in several major neurodegenerative disorders associated with parkinsonism.¹⁰ In combination, these biomarkers convolute the clinical pathological spectrum of ALS/PDC, and, as a result of the heterogeneity, ALS/PDC can only be confirmed by postmortem examination.⁴

Genetic studies posit that ALS/PDC does not follow Mendelian patterns of inheritance.¹ Rather, it follows an irregular, multifactorial autosomal dominant mode of inheritance¹¹ with incomplete penetrance.¹² The familial nature of ALS/PDC indicates that one or more genes may be responsible; however, attempts to identify a causative gene have yet to be successful.⁹ Since NFTs are the most prominent biomarker of this disease, a genetic study of Guam focused on the gene that encodes for microtubule-associated protein tau (MAPT). Two independent single nucleotide polymorphisms (SNPs), variations of a single base pair in a DNA sequence, within the MAPT region confer a risk of susceptibility by a recessive, cis-acting mechanism; however, the polymorphisms only increase the risk in combination with other genetic and environmental factors.¹³

Methods

A computer literature search of the PubMed/MEDLINE, Google Scholar, and Mendeley databases was conducted to find relevant literature on ALS/PDC in the Western Pacific foci with respect to cycad and mineral neurotoxicity. The main search terms were ALS/PDC, cycad, iron, manganese, Guam, Kii Peninsula, West Papua New Guinea, and neuro# (the symbol is used for identifying all words starting with neuro, e.g. neurodegenerative, neurotoxic, and neuropathological). The literature found to

satisfy the following criteria was included in the review: (a) examination of at least one risk factor of ALS/PDC in at least one of the Western Pacific foci; (b) discussion of cycad or mineral neurotoxicity; and/or (c) discussion of the biomarkers of ALS/PDC. The following literature was excluded: (a) language other than English, (b) small sample size, and/or (c) ALS/PDC not being the central focus.

Results

Environmental Aspects

Although genetic factors may be linked to ALS/PDC, the decline in prevalence of these disorders over a short period argues for a gene-environmental interaction in which exogenous or environmental factors may contribute to the pathogenesis of ALS/PDC. Many environmental risk factors have been examined over the past years, including exposure to animals, fish poisoning, and mineral deficiencies; however, no relationship has been definitively identified. There are two major environmental hypotheses regarding ALS/PDC that are typically researched separately: the vegetal hypothesis and the mineral hypothesis.

1. Vegetal Hypothesis

The vegetal hypothesis focuses on a common, etiological factor to all three ALS/PDC foci: the exposure to traditional foods and medicines derived from the cycad plant.¹⁴ Major cycad neurotoxins correlated with a high incidence of ALS/PDC include methylazoxymethanol β -D-glucoside (cycasin), and its aglycone methyl-azoxymethanol acetate (MAM), β -N-methylamino-L-alanine (BMAA), and β -oxalylamino-L-alanine (BOAA).¹⁵ The toxic part of cycasin is the active ingredient that is released as MAM by enzymatic processes occurring in digestion; thus, cycasin only exerts a toxic effect when it is ingested.¹⁶

The most affected population, due to consumption of cycads and inhalation of cycad pollen, was the indigenous Chamorro of Guam. They used fresh cycad seed cover to relieve thirst and dried seed cover as a confection;¹⁷ however, the most studied and common traditional food of the Chamorro is a flour called *fadang* made from the seed. Although the preparation of the flour includes successive washings of cycad ovules to reduce the content of cycad toxins, a study revealed large concentrations of cycasin were still present in the flour which, though not

lethal, did induce acute illness in children likely due to the hepatotoxic properties of cycasin.¹⁸ Additionally, the consumption of flying foxes, or fruit bats, in the diets of the Chamorros has been proposed to cause ALS/PDC due to the bats' substantial consumption of cycad seeds and bioaccumulation BMAA.¹⁹ It should be noted, however, that flying foxes are not part of the diet of Japanese or New Guinean subjects at risk for ALS/PDC. Moreover, cycads have been observed to produce pollen with high concentrations of cycasin and BMAA.²⁰ The respiratory system is another potential entry path for cycad toxins: the pollen contacts the nasal epithelium and can be transported to brain tissue to induce neurotoxic effects.²¹ A recent study confirmed that intranasal administration of MAM in mice caused elevated mitogen-activated protein kinases (MAPKs) and increased caspase-3 activity, which are linked to the tau aggregation and neuronal cell death that is characteristic of ALS/PDC.²²

The medicinal use of cycads through prolonged subcutaneous or repeated oral application of raw cycad seed is common to all three foci of the Western Pacific. The cycad seed has been used as a topical treatment for skin lesions,^{23, 24} but such use undoubtedly declined as man-made pharmaceuticals were introduced. The use of the cycad seed for oral medicine was practiced in Japanese folk medicine in the Kii Peninsula until the 1980s, with prescriptions written by practitioners and filled by pharmacies.²⁴ It should also be noted that the Fore people, outside of the ALS/PDC foci, living in the south-eastern Papua New Guinea, were exposed to cycad toxins by chewing the fleshy cycad seed cover and spitting the contents into food which precipitated kuru, a neurodegenerative disease with tau pathology.⁵

Despite extensive research on the cycad, no conclusive association between ALS/PDC and plant or animal toxins has become evident. A study on cycad-derived products such as *fadang*, flying foxes, and topical medicine as possible risk factors for dementia, mild cognitive impairment (MCI), and ALS/PDC found no significant relationship between the consumption of flying foxes or topical medicine, but did find a significant odds ratio (OR), which provides a measure of the strength of association,²⁵ for picking, processing, and eating *fadang* in young

adulthood for any of the neurodegenerative diseases present in the native population of Guam.²⁶ Although starch-making from cycads was prevalent in the Mariana Islands, ALS/PDC was found to be concentrated only in certain villages on Guam such as Umatac, Merizo, and Inarajan; however, the BMAA content of cycad samples from Umatac contained no significant differences relative to the controls.²⁷ Additionally, a survey of the Hohara area of Nasei-cho, one of the foci in the Kii Peninsula, showed no relationship between cycad use and neurological disease.²⁸ Furthermore, the consumption of cycads is not remarkable because aboriginal groups in Australia historically prepared food from carefully detoxified cycad seed ovules, and Japanese living in the Ryukyu Islands employed fermentation techniques to eliminate cycad toxins without precipitating neurological disease.²⁹ Thus, the vegetal hypothesis by itself appears to lack scientific support, suggesting the role of other possible risk factors.

2. Mineral Hypothesis

Environmental data from the Western Pacific endemic foci of ALS/PDC supports the interactions between essential and neurotoxic metals and contributes to what is known as the mineral hypothesis. Although ALS/PDC is possibly associated with a constellation of metals,^{30, 31, 32, 33} this review focuses on the bioaccumulation of iron (Fe) and manganese (Mn) from the environment in bulk central nervous system (CNS) tissue of patients in the ALS/PDC foci. Metals and trace elements play salient roles in the CNS; however, clinical disease may result from deficiencies and excesses of such essential minerals, and nonessential trace elements may also induce neurological disease through excessive exposure.³⁴ Thus, iron and manganese may be causally implicated in ALS/PDC in the Western Pacific foci based on the bio-accumulation of neurotoxic minerals in the soil, drinking water, and vegetation.

A. Iron

Iron is integral to many biological functions: it has a role in many enzymes involved in oxidative and amino acid metabolism, it has an effect on dopamine D2 receptor function, and it interacts with other neurotransmitters such as gamma-aminobutyric acid (GABA)³⁵ and glutamate.³⁶ Iron deposition in the brain is most prominent in the globus pallidus, red nucleus, substantia nigra pars reticulata,

putamen, caudate, and the dentate nucleus, but is found in white matter and cortex as well.³⁷ Trace amounts of these deposits are minimal at birth and gradually increase for the first three decades of life after which they tend to stabilize until about the sixth decade of life, and then insidiously increase.³⁸ Excessive iron deposition is associated as a putative factor in the pathogenesis of neurodegenerative disorders, most notably AD and Parkinson's disease (PD).³⁹

The neurotoxic effects of iron may result from iron catalyzing the production of reactive oxygen species (ROS) through the Fenton and Haber-Weiss reactions, provoking oxidative stress.^{40, 41} Moreover, the products of these reactions can continuously form organic free radicals, spawning a self-perpetuating neuronal death cascade that is "continuously propagated" by excess free iron.⁴²

A neutron activation analysis (NAA), a non-destructive technique for simultaneously determining the concentrations of trace elements in a sample,⁴³ of iron and zinc (Zn) in gray and white matter of the frontal and occipital regions in Guam patients with ALS/PDC indicated an increase of iron in gray and white matter and a decrease of zinc in gray matter, relative to controls, coupled with an excess of bioavailable aluminum (Al) and deficiency of calcium (Ca).⁴⁴ However, this result conflicts with the findings of another study of Guamanian patients with ALS/PDC: eight metals in formalin-fixed brain tissue were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS),⁴⁵ revealing that for all metals, the concentrations tended to be higher in gray matter than in white matter, and finding no significant differences between the patients and the control groups for iron.³⁰ Even though the sample sizes of both studies are small, the contradictory results of the two studies suggest other risk factors are at play in the precipitation of ALS/PDC, in addition to iron concentration.

In an environmental field study, samples of soil, water, and vegetation were obtained from three southern villages of Guam with high incidences of neurodegenerative disease — that is, Umatac, Merizo, and Inarajan — to investigate any abnormal mineral concentrations and whether they could be linked to ALS/PDC. The study indicated higher levels of iron, among other compounds, in these villages

than in the disease-free north of Guam. Specifically, elevated levels of iron were found in the red laterite top soils along the western side of Guam,⁴⁶ in the river water at Merizo, and in the vegetation around Umatac and Merizo.³¹

The soil, drinking water, and vegetation were also analyzed for mineral imbalances in the Kii Peninsula. An analysis of seven metal concentrations in the environment of the Hohara area reported elevation of iron and manganese in the drinking water of Iseji, one of the five sub regions of Hohara, relative to that of Uchikawame and Nankohdai, two control areas remote from the focus.⁴⁷ In contrast, in another study, a chemical analysis by neutron activation of Guam and Kii Peninsula water sources found no significant difference in iron content;⁴⁸ however, a constellation of factors, such as a deficiency in suitable controls, may result in inconsistencies across studies that may be related to the incidence of ALS/PDC in the foci.

B. Manganese

Manganese is another essential metal that is important for many physiological processes such as carbohydrate metabolism, calcium absorption, defense against free radicals, and is an important cofactor in several enzymes integral for neuronal and glial cell function and enzymes involved in neurotransmitter synthesis and metabolism, namely dopamine, GABA, and glutamate.^{49, 50, 51} Despite its vital role in a multitude of biological functions, excessive manganese exposure is associated with several neurodegenerative diseases, including ALS, PD, Manganism (manganese poisoning, an analog of PD), and AD.⁵²

The highest concentrations of manganese occur in the basal ganglia, more specifically, the same deep-brain nuclei associated with iron deposition.^{53, 54, 55, 56} Due to the similarity of iron and manganese, both metals are interdependent and can use the same transporters.⁵⁷ Moreover, the neurotoxic effects may transpire from the interactions between iron and manganese: an *in vivo* study indicated elevated manganese exposure facilitated unidirectional influx of iron from the blood to the cerebrospinal fluid (CSF) in rats, thus by increasing free iron levels, manganese may elicit iron-induced oxidative stress and cause oxidative damage to neurons.^{58, 59} Additionally,

excess manganese has been linked to decreased function of dopamine, glutamate, and GABA which can induce neurological disease.⁶⁰

By neutron activation analysis, a study of Guam and Kii Peninsula ALS-PDC analyzed samples of water, soil, plants, CNS tissue, and cattle hair. The study found a higher content of manganese in the spinal cord than in any other CNS tissue and, concurrently, reported a generally high content of manganese in the river and drinking water of Guam, particularly in Inarajan and in the tap water of Agana. In addition, while the water samples from the Kii Peninsula have about the same content of manganese as the rivers in the Kinki District and the rest of Japan, the residences of a few patients showed a relatively high content of manganese in their drinking water. Furthermore, the study found elevated manganese levels in the soil taken from both foci, and significantly high manganese content in the hair of cattle living in the Kii Peninsula.⁶¹

The same environmental study of soil, water, and vegetation in three high incidence villages of Guam — Umatac, Merizo, and Inarajan — found elevated manganese levels, along with iron and other metals, in the top soils and in the vegetation around Umatac and Merizo. The results suggest that the elevated levels of iron and manganese, besides other metals, in the soil could cause enhanced levels of magnetic susceptibility in Southern Guam which may be a key to understanding the pathogenesis of ALS/PDC.³¹

Samples of soil, water, and vegetation were also studied in the Kii Peninsula in which the concentrations of seven minerals were analyzed. The study found significantly higher manganese levels in the paddy field soils of Iseji and higher manganese and iron levels in Iseji drinking water, relative to the two control areas Uchiwakame and Nankohai. Furthermore, the study found higher manganese intake in Iseji local rice consumers than in imported rice consumers from the same area and in three control areas (Kiri-hara, Uchiwakame, and Nankohai), and higher manganese content on a dry-weight basis in boiled rice in Iseji than in Kiri-hara. Regardless of the types of samples collected, the manganese content was always more elevated in Iseji than in the control areas.⁴⁷

In West Papua New Guinea, the primitive Auyu and Jakai (Jaqai) populations lacked manufactured products due to their isolation and primitive technology.⁶² The high ALS/PDC incidence in this focus was hypothesized to be associated with low concentrations of calcium and magnesium (Mg) in their drinking water,⁶² which is also seen in other foci.^{63, 64} However, ALS prevalence in these sessile populations declined without any known change in their source of drinking water.³ This finding conflicts with a study of the Kii Peninsula that showed an increase in ALS incidence due to a change in sources of drinking water.⁶⁵ Contradictory evidence may suggest the role of other risk factors and calls for further investigation.

Concluding Remarks

Although there are studies focusing on the possible risk factors in the three foci of the Western Pacific, they are often insufficient in suitable controls, and, in some cases,

sample size to show that the cycads and the levels of metals are causally related to the high incidence of ALS/PDC. While these studies tend to treat the vegetal and mineral hypotheses as mutually exclusive, it may be more beneficial to consider an intersectional relationship. For instance, the vegetal hypothesis focuses on cycads, yet, the Western Pacific foci is known to be a manganese-rich environment (as is plainly evident in the aforementioned studies), especially as it relates to Guam,⁶⁶ with elevated levels of iron and low levels of calcium and magnesium present, suggesting possible mineral interdependency^{57, 59} and antagonism,^{67, 68, 47} respectively. Thus, for the reasons discussed *supra*, further investigation of cycads and metal neurotoxicity, in combination, in all three foci of the Western Pacific, is warranted, and could be beneficial in further understanding the etiology and underlying mechanisms of the enigmatic ALS/PDC endemic.

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Does Napping Boost Benefits of Brain-Training for Working Memory?

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ABSTRACT

Working memory (WM) is engaged in most cognitive tasks deployed in the human brain. Brain-training regimens that target WM may promote plasticity, leading to improved WM skills. Additionally, sleep is known to facilitate consolidation of newly learned information and skills. Here, we asked if napping could boost benefits of brain-training for WM. Participants completed ten days of WM training on an N-back task; on each training day, a subset of participants were given a 30-minute nap opportunity (with EEG recording) immediately following their training session (training+nap). In Study 1 (n=10), we equated the amount of training (20-min training/day) in all participants and compared training only to training+nap. In Study 2 (n=8), we asked if napping can effectively replace additional time spent training; we compared training+nap (20-min training/day) to double training (40-min training/day). On average, the nap group slept 16.0±5.77 minutes/nap in Study 1 and 15.98±7.44 minutes/nap in Study 2. Our dependent measure of performance was the highest N-level achieved on each day of training. In both studies, we found that performance improved across the ten days of the study. However, there was no day x group interaction in either study, suggesting that the degree of improvement did not differ between training only vs. training+nap groups. In Study 2, there was a trend towards more improvement with double training compared to single training+nap. For people looking to dedicate time each day to improving their WM, it may be more beneficial to spend the entire time training rather than training+napping.

Keywords: brain-training, video-games, working memory, neuroplasticity, sleep, N-back



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Professor Aaron Seitz is an internationally recognized expert on the mechanisms of learning and memory using behavioral, computational and neuroscientific methodologies. His research over the last 15 years has focused on mechanisms of plasticity and learning in the sensory/perceptual systems. A key aspect of his recent research is applying knowledge of plasticity mechanisms in the brain to create brain-training video games that are effective in improving performance in real-world tasks. A notable example is his vision training game ULTIMEYES that leads to vision improvement that positively transfers to on-field performance in baseball. He is now the Director of the newly founded UCR Brain Game Center for Mental Fitness and Well-being that has the mission to research, test, and 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.

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INTRODUCTION

Methods for improving memory have been a primary focus in cognitive psychology and neuroscience for many decades. Though research has made great strides in understanding memory domains and mechanisms, developing a reliable way to improve memory has proved challenging. Recently, working memory (WM) has been targeted as a promising domain for improvement. WM is a cognitive system concerned with temporarily holding information for immediate use. WM underlies, and interacts with, many other cognitive systems, including long-term memory and our ability to reason, comprehend and learn. Therefore, improving WM capacity (i.e., the amount of information that is temporarily stored for immediate use) and accuracy (i.e., ability to correctly remember such information) could yield benefits across many cognitive domains (Deveau et al., 2015). Since WM plays an interactive role in other cognitive domains, improving this facet could therefore improve cognition and help combat cognitive deficits faced with age. Research has indicated that WM is a plastic domain that can be strengthened with more practice (Klingberg, 2010).

Brain plasticity (i.e., neuroplasticity) is the brain's ability to change and/or strengthen connections based on use of specific brain regions or populations of neurons. Though WM was initially considered to be a "non-plastic" domain, unable to improve or weaken, recent studies have found the opposite is true (Klingberg, 2010). WM can be strengthened; improvements in WM are associated with the frontal and parietal regions of the brain (Thompson & Waskom, 2016). Specific regions associated with WM improvement are the executive function region of the brain (pre-frontal cortex) and the attentional network (dorsal parietal cortex) (Thompson & Waskom, 2016).

Previous studies have primarily utilized N-back tasks in order to train WM (Jaeggi et al., 2008; Smith et al., 2009; Buschkuhl and Jaeggi, 2010; Thompson & Waskom, 2016). The term "N-back" refers to how many objects back the test-taker is required to match their response with. The most common utilization of N-back training constitutes recalling shapes of different colors a certain number of screens (N) back (Figure 1B). Conversely, Thompson & Waskom (2016) implemented a different N-back

task consisting of auditory and visual cues of consonant letters spaced in peripheral regions of a computer screen. Regardless of discrepancy in N-back task arrangement, this task has proven to be a useful tool in increasing working memory abilities on the specific task.

More research is required to extend these WM improvements to a more accessible and generalized platform. Research into creating an accessible tool for everyday use has led to development of applications aimed at exercising cognition, so-called brain-training games. Brain-training games are intended to improve cognitive functions and hopefully generalize to be to domains not specifically trained (Green & Seitz, 2009). Based on previous findings focused to improve WM, increased cognitive training in the WM domain not only yields improvement in task but has also led to altered neural networks causing an expansion in WM capacity (Thompson & Waskom, 2016).

Post-learning sleep has been shown to facilitate plasticity and improve behavioral performance in a wide-range of memory domains (Diekelmann & Born, 2010). However, the impact of sleep on WM has mostly been studied in the context of WM deficits due to sleep deprivation or sleep disorders (Mednick et al., 2002). One domain that has shown a benefit of sleep, and might share general learning mechanisms with WM, is perceptual learning (Deveau et al., 2015; Mednick et al., 2003). Perceptual learning is improved performance on a sensory task, typically following training or practice. Visual perceptual learning is vulnerable to deterioration from over-training within a session or day (Mednick et al., 2002, 2005); however, sleep, including short periods of sleep (i.e., napping), can recover performance and lead to performance gains without additional training (Mednick et al., 2003). This suggests that sleep works to promote experience-dependent changes in brain plasticity induced by training.

Here, we aim to test if this benefit of napping extends to plasticity induced by WM training. In the current study, we examined two main questions: Does training+napping facilitate the rate of WM improvement across ten days of training (when time spent training is held constant)? And can napping replace additional time spent training (in other words, is 20min of training plus 20min of napping

just as effective as 40min of training)? We hypothesized that greater WM improvements would be elicited by training+napping than by training alone, and that napping would impart the same amount of WM benefit as additional time spent training.

METHODS

Participants

A total of 19 participants (8 female) between ages 18-30 (19.94 ± 1.68 years old) were recruited through an email invitation sent to students at the University of California, Riverside (UCR). Interested individuals responded to the email with their availability; Those who were available every weekday for at least 90-minutes between the hours of 11am-4pm were then invited to meet with the researchers to learn more about the study. Eligibility requirements included refraining from caffeine and alcohol consumption the morning of each study day. Each participant signed a written consent form to participate in the experiment, which was approved by the Human Research Review Board at UCR.

Protocol

The WM training regimen involved performing an N-back WM task each day for a total of 10 days (excluding weekends). Time-of-day of training was not strictly controlled, but all training sessions were completed between the hours of 11am-4pm. Participants were assigned to either a training-only or a training+nap group. In Study 1, all participants completed one, 20-minute WM training session per day (single training). Following the training session, participants in the training-only group were allowed to leave the lab. Participants in the training+nap group had EEG electrodes attached (~15 min), followed by a 30-minute opportunity to nap. In Study 2, the nap+training group followed the same procedure; however, the training only group completed two 20-minute training sessions per day (double training), with a 5 minute break between sessions.

Working Memory N-Back Task

In this study, participants completed a common WM task called the N-back task (Jaeggi et al., 2008; Smith et al., 2009; Buschkuhl and Jaeggi, 2010). The task was performed on an iPad using an application developed for this study.

An experimental *trial* consisted of three separate stages: the response, feedback and inter-stimulus stages (Figure 1A). In the response stage, a colored object was displayed on screen and participants were given 2500 milliseconds (ms) to determine whether or not it matched the object shown “n” trials back. If there was a match, participants responded by tapping the screen. After the response stage, participants saw a 300 ms feedback window where the shape was circled in green for a correct response or in red for an incorrect response (Figure 1B). Following the feedback stage, a grey object appeared on screen for 200 ms during the inter-stimulus stage. This was meant to reset the trial before presenting a new colored object. The overall trial was 3000 ms in length.

An experimental *block* consisted of 40 trials with the same “n” level. The difficulty of sequential blocks was adaptively adjusted during the session based off a participant’s performance. This is a common method used in the N-Back Task; however, different studies have differing thresholds for level changes (Harbison et al., 2011). In our study, if a participant achieved 80% correct or above on a given block, the task became harder with a +1 increase in “n” level. If a participant achieved 50% correct or below on a given block, the task became easier with a -1 decrease in “n” level. If a participant achieved between 50% and 80% correct, the task difficulty and “n” level remained the same. The highest “n” level achieved for each participant on each day of training was our measure of WM performance.

Polysomnography (PSG)

PSG data was acquired using with Ag/AgCl electrodes placed according to the international 10-20 System (Jasper, 1958). We recorded electroencephalogram (EEG) from scalp electrode sites C3, C4, O1 and O2, as well as an on-line common reference channel (FCz location). Additional channels included two mastoids for offline re-referencing (A1 and A2), two electrooculogram (EOG), and one ground. Recordings were sampled at 500Hz.

Offline, EEG and EOG data were re-referenced to contralateral mastoids and filtered between 0.3 Hz and 35 Hz. A 60 Hz notch filter was also used to eliminate potential background noise. Data was visually scored in 30-s epochs according to the sleep staging criteria of Rechtschaffen

and Kales (1968). Sleep architecture variables included minutes and percentage of Stage 1, Stage 2, slow wave sleep (SWS), and rapid eye movement (REM) sleep, as well as total sleep time (TST), sleep latency (SL), and sleep efficiency (SE).

Statistical Analyses/Data Reduction

Mixed-model analysis of variance (ANOVA) was utilized to examine performance across the ten days of the study in our experimental groups. In these analyses, Day (1-10) was our within factor, and group (training-only vs. training+nap) was our between factor. Independent-sample t-tests were used to examine group differences at individual timepoints of interest. Repeated-measures ANOVA tested for changes in nap total sleep time across the 10 naps.

One participant (Study 1) was removed from data analyses due to receiving double training instead of single training on half of the study days. Of the total 100 naps recorded across studies 1 and 2, 4 nap records were missing due to technical error. Behavioral data from these days are included in the analyses; however, sleep stage variables were unavailable and were treated as missing. Sleeping throughout the entire 30-minute nap opportunity was not

required and not all nap group participants slept during every nap opportunity; days where participants were unable to sleep were still included in the analyses. Our final sample size for each study is as follows:

Study 1 (single training: $n = 4$, training+nap: $n = 6$) and Study 2 (double training: $n = 4$, training+nap: $n = 4$). For participants assigned to double training in Study 2, we calculated their highest N-level per day by averaging the highest N-level achieved in each of their 2 sessions/day.

RESULTS

Nap Results

People were able to nap given a 30-minute opportunity. Sleep descriptives, including minutes spent in each sleep stage, can be found in **Tables 1 and 2**. In Study 1, TST did not significantly vary across the 10 naps [$F(9,27)=0.704$, $p=0.7$] (**Figure 2A**). Conversely, Study 2 showed a significant increase in TST across naps [$F(9,18)=3.59$, $p=0.01$] (**Figure 2B**).

Behavioral Results

In Study 1, participants were assigned to either a single training or training+nap group. Over the course of ten days of training, the single training group completed

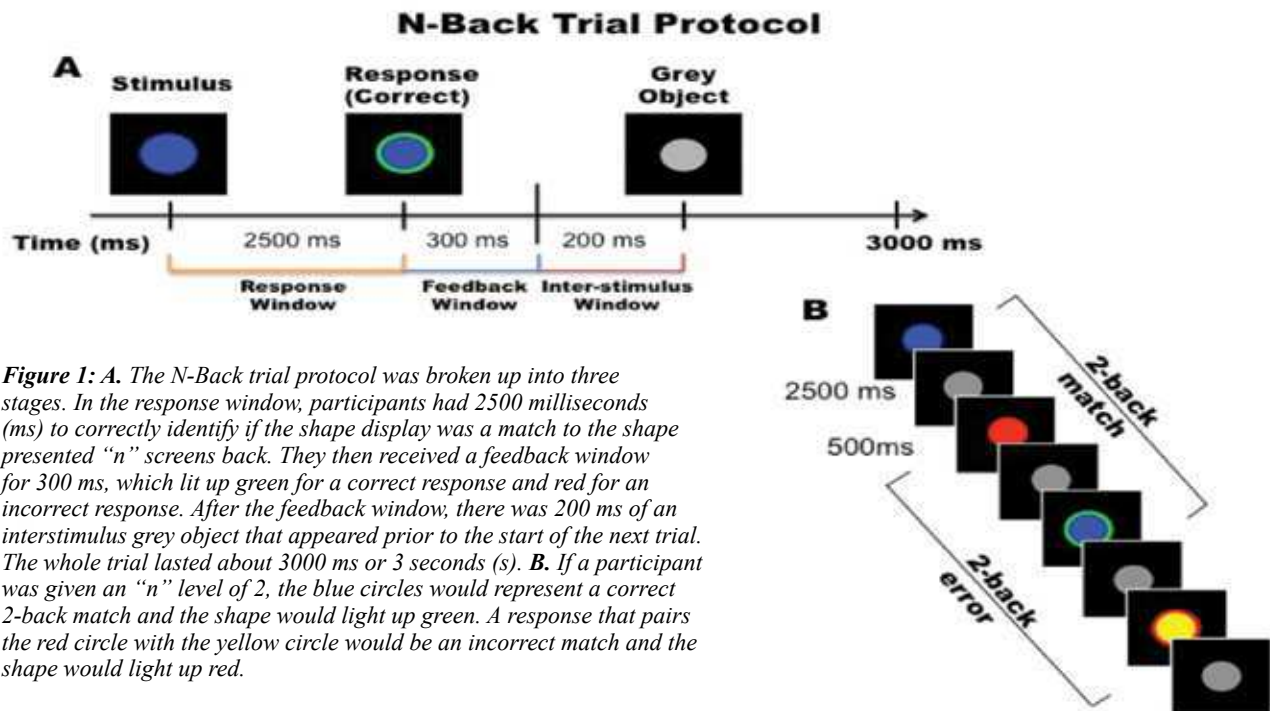


Figure 1: A. The N-Back trial protocol was broken up into three stages. In the response window, participants had 2500 milliseconds (ms) to correctly identify if the shape display was a match to the shape presented “n” screens back. They then received a feedback window for 300 ms, which lit up green for a correct response and red for an incorrect response. After the feedback window, there was 200 ms of an interstimulus grey object that appeared prior to the start of the next trial. The whole trial lasted about 3000 ms or 3 seconds (s). B. If a participant was given an “n” level of 2, the blue circles would represent a correct 2-back match and the shape would light up green. A response that pairs the red circle with the yellow circle would be an incorrect match and the shape would light up red.

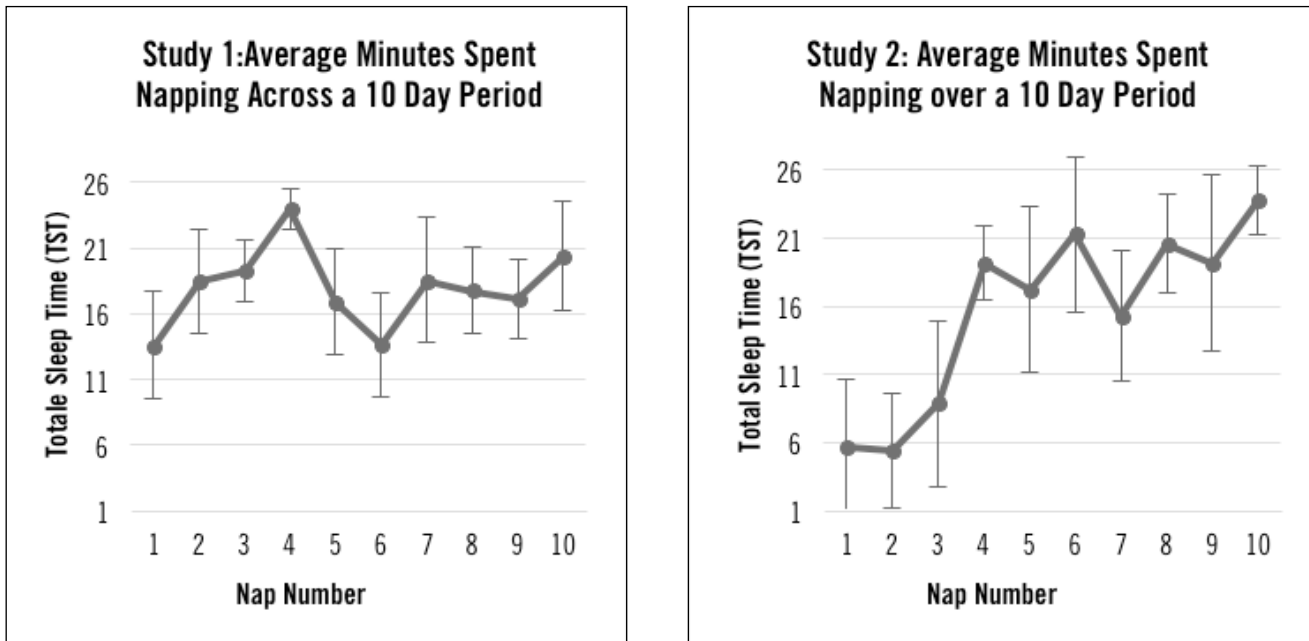


Figure 2- Average Total Sleep Time Per Nap

(A) In Study 1, there was no overall change in total sleep time across ten naps. (B) In Study 2, total sleep time increased across ten naps. Error bars represent +/- 1 standard error of the mean.

	TST	Stage 1	Stage 2	SWS	REM	Sleep Efficiency
Mean	16.34	4.91	9.66	1.77	0	54.39
Std. Deviation	5.77	1.83	4.33	2.33	0	19.60
Minimum	6.56	2.83	3.72	0	0	20.98
Maximum	21.00	8.00	15.90	4.95	0	70.35

Note: TST = total sleep time; SWS = slow wave sleep; REM = rapid eye movement; Sleep efficiency was calculated as TST/time in bed. Besides sleep efficiency (which is a percentage), the units of all other variables are minutes.

Table 1- Sleep Descriptives for Study 1

	TST	Stage 1	Stage 2	SWS	REM	Sleep Efficiency
Mean	15.98	4.76	8.59	1.95	0.69	52.92
Std. Deviation	7.44	3.05	4.14	3.36	0.85	23.75
Minimum	4.95	1.70	3.25	0	0	17.95
Maximum	20.72	8.50	13.00	6.95	1.75	69.12

Note: TST = total sleep time; SWS = slow wave sleep; REM = rapid eye movement; Sleep efficiency was calculated as TST/time in bed. Besides sleep efficiency (which is a percentage), the units of all other variables are minutes.

Table 2- Sleep Descriptives for Study 2

a total of 88.8 ± 6.4 (mean \pm SD) blocks of training, and the training+nap group completed 90.7 ± 5.8 blocks of training. A Group x Day mixed-model ANOVA showed a significant main effect of Day ($F(9,72)=10.66, p<0.001$), indicating that overall, participants showed improvement in highest N-back level reached over the 10-day training period (**Figure 3A**). There was no main effect of Group ($F(1,8)=1.05, p=0.34$), and the Group x Day interaction was also non-significant ($F(9,72)=1.48, p=0.17$). The lack of a significant interaction suggests that both groups improved along a similar trajectory. Thus, we did not find evidence that 30-minutes of napping facilitates training-induced WM improvements.

In Study 2, participants were assigned to either a double training or training+nap group. Over the course of ten days of training, the double training group completed 292.8 ± 13.0 (mean \pm SD) blocks of training, and the training+nap group completed 151.5 ± 11.8 blocks of training. Similar to Study 1, participants showed overall improvement in highest N-back level achieved over the 10-day period ($F(9,54)=4.24, p<0.001$, (**Figure 3B**). There was no main effect of Group ($F(1,6)=2.11, p=0.20$), and the Group x

Day interaction was trending, but did not reach traditional levels of significance ($F(9,54)=1.87, p=0.08$). The general pattern of results show that following training day 4, the double training group was numerically better than the training+nap group. By Day 10, the double training group was significantly better than the training+nap group ($t(6)=3.61, p=0.01$, Cohen's $d=2.55$). From this result, we can conclude that 30 minutes of napping does not replace a second training session. If anything, double training appears to be on a trajectory towards showing significantly greater WM improvements than training+napping.

DISCUSSION/CONCLUSION

The common goal of study 1 and study 2 was to determine if napping can facilitate training-induced improvements in WM. Study 1 compared 20-minutes of training plus a short nap to training alone; Study 2 compared 20-minutes of training plus a short nap to 40-minutes of training alone. We did not find a benefit of napping in either study. Additionally, the results of Study 2 suggest that the most effective training regimen might be double training. In other words, if you spend 40-minutes a day trying to improve your WM, that time might be best spent training

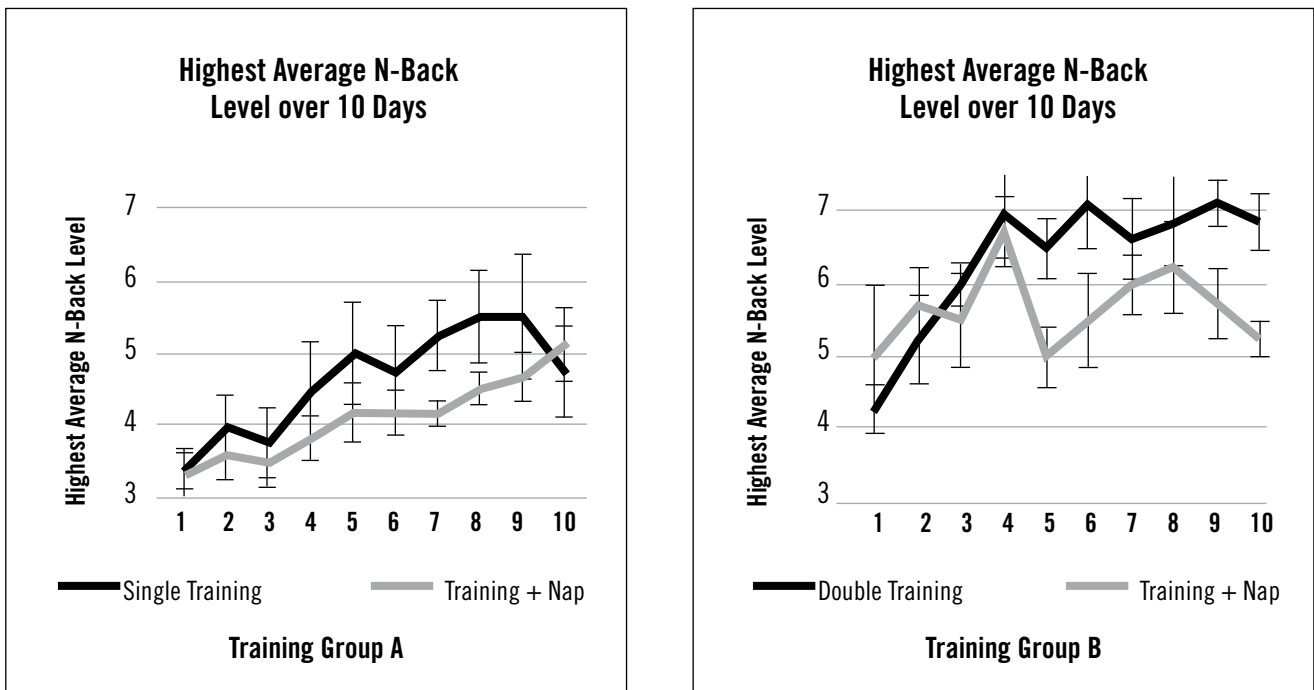


Figure 3 - Highest N-level Across 10 Days of Training for Study 1 and Study 2
 (A) In Study 1, the single training and training+nap groups show similar trajectories of improvement across ten days of training.
 (B) In Study 2, the double training group shows a trends toward more improvement than the training+nap group.

the entire time rather than training and taking a quick nap.

Both studies found an improvement in N-back task performance over the ten days of training. This supports previous findings that WM can be improved with practice (Klingberg, 2010). However, the current study only examined performance on one specific task. An important extension of this work will be to test if N-back training can lead to generalized improvements on other working memory tasks and other cognitive domains.

Neither Study 1 nor Study 2 found evidence that napping boosted training-induced WM gains. It is possible that WM is not a cognitive domain consolidated or strengthened by sleep. However, it is important to acknowledge that all participants did have sleep between days – at night – and these results do not conclusively eliminate sleep as a factor in the improvement we saw across days. Rather, we can only conclude that a short nap (~15 min) did not facilitate WM improvements. Another possibility is that the nap was too short to elicit sleep-related benefits. In general, research on napping and memory typically utilizes longer napping periods (60-90 minutes) in order to capitalize on potential benefits from all sleep stages in a sleep cycle (Mednick et al., 2003). For example, perceptual learning gains are typically seen in conditions where the naps include both slow wave sleep (SWS) and rapid eye movement (REM) sleep (Mednick et al., 2003). In the current study, our naps were predominantly composed of lighter Stage 1 and Stage 2 sleep, and had very little or no SWS or REM. Another caveat is that WM performance was

not assessed immediately following the nap. Therefore, it is still possible that napping could boost same-day WM performance, perhaps by reducing fatigue. We can only conclude that napping did not benefit across-day WM performance. Future studies should investigate the impact of longer naps on training-induced WM performance and the relation between specific sleep stages/features and WM performance.

Another limitation of this study was the small sample size. This was due to space and time limitations - we could only have so many participants nap in the lab during designated napping hours each day. A larger sample size would give us the ability to examine factors related to individual differences that may be critical to understanding interactions between napping and WM training.

Overall, WM training may not be facilitated by a 30-minute nap opportunity. Rather, people who are looking to improve their WM might be better off investing the extra time doing additional training, not napping. Future work still needs to establish if training-induced WM improvements generalize to other tasks and cognitive functions. If WM brain-training does generalize to overall WM improvements, a viable outlet to improve cognition and battle degenerative deficits could be accomplished. In all, the study at hand showcases the potential benefits of WM training and demonstrates that this is a promising area of research for developing tools and strategies to counteract cognitive deficits associated with reduced WM abilities.

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The Differences in STEM Feelings and Interest Between Boys and Girls

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ABSTRACT

Children are exposed to many areas of interest and careers through accessible media and technological devices. Research has shown that STEM careers are lacking in female representation. According to the National Science Foundation, women only represented 28% of individuals in science and engineering occupations in 2010 (NSF 2014). Exposure to STEM careers in early childhood may be an underlying cause of this underrepresentation; thus considering young children's feelings and interest in STEM is important for nurturing students to enter STEM fields. Children between ages 3.61 to 7.21 years ($N = 79$) were asked about their interests in STEM activities and feelings about a STEM task before and after playing a STEM application. Children reported decreased levels of STEM interest from pretest to posttest, whereas children's self-efficacy for a STEM activity did not significantly differ from pretest to posttest. The results suggest that short-term exposure to a STEM application did not increase children's STEM interest and self-efficacy toward STEM, as measured by children's verbal report.

Keywords: STEM diversity, Feelings, Self-Efficacy, Interest, Children, STEM differences



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INTRODUCTION

In the 2014 National Science Foundation's (NSF) Science and Engineering Indicators report, American society's negligence to nurture girls' interests in STEM was considered as one of the possible underlying causes of lesser female representation in Science, Technology, Engineering, and Mathematics (STEM) fields (National Science Board, 2014). Understanding early childhood experiences that influence gender differences in feelings and interests toward STEM can bring to light this inequality and contribute to understanding how to increase female representation.

A factor that has been found to have one of the strongest influences on children's feelings and interests in STEM is their exposure to or experiences with STEM (Meluso, Zheng, Spires, & Lester, 2012). Some children have positive feelings while others have negative feelings toward STEM (Cvencek, Meltzoff, & Greenwald, 2011). Researchers have posited that girls may experience lack of positive feelings toward STEM, which may result in girls' negative perception of STEM (Buck, Cook, Quigley, Eastwood, & Lucas, 2009). Moreover, in order to maintain and promote high interest in STEM, children must have a sense of connection with their envisioned careers (Kleinfeld, 2001). As girls' feelings toward STEM become well rooted and solidified, their interests may also increase (Master, Cheryan, & Meltzoff, 2016). Prior research has suggested that it is important to discover the ways in which girls can have positive feelings and interests in STEM-related activities.

Given the considerable amount of time young children spend with media (Richert et al., 2011), young children's feelings and interest toward STEM could be supported with the use of and exposure to technology and media. Some research has suggested that digital games are able to help children make analogies between the game and the real world so that they can better understand real world concepts. For example, Gros (2007) found that both male and female students who were given games to supplement the material taught in school could relate and connect concepts introduced in the game to lessons taught in class. A drawback from using the games was that students required a large amount of time to absorb the content from

the game, guidance, and reassuring support from teachers for game instructions and higher transference of learning.

In a study with secondary school students, Miller et al. (2011) found children significantly gained content knowledge in STEM. Miller et al. (2011) selected children and randomly assigned them to play one of three different cases of Crime Scene Investigation. The data revealed that previous experience with web-based forensic science games was significantly related to performance on the pre- and post-exposure content knowledge tests (Miller et al., 2011). It could be that the forensic science games were straightforward and children had no difficulty learning the game. The current study uses a STEM task that is more challenging for children, so prior exposure to STEM concepts may be helpful in solving the activity. In addition, Miller et al. (2011) reported that participants who did not find the science games challenging were likely to learn more from the games than other participants who did find the games challenging. Participants' motivation to fulfill a STEM career was also directly proportional to their satisfaction of the game (Gros, 2007).

To provide children with positive feelings about STEM activities, it is important to understand conditions that help children to feel secure about their interests, experiences, ideas, and emotional responses to STEM. Exposure to STEM tablet games may increase children's feelings and interest toward STEM, and may provide more supportive data of media usage as an influential component in increasing children's feelings and interests in STEM (Meluso, et al. 2012). However, little research has been conducted to document the feelings about and interest in STEM in young children between ages 3 to 8. Further, prior studies have not focused on the comparison between males' and females' feelings and interests toward STEM after some exposure to STEM games (Yazilatas, Svensson, Vries, & Saharso, 2013). The current study examined the effects of children's exposure to STEM concepts and their performance on STEM tasks without additional guidance or feedback as in Gros (2007) to prevent teaching children the correct solution. Rather, the study was designed towards the interest of allowing participants to elicit their interests and feelings for certain STEM concepts.

The aim of the current study is to understand children's feelings and interest toward STEM and the transfer of learning from an interactive digital game to a real-world problem. Participants played an iPad game that was designed to teach a STEM concept and were asked to use that knowledge to complete a real-world task involving similar materials presented in the game. The study measured children's exposure to STEM using open-ended questions, children's pre and post-task reported feelings and interests toward STEM, and children's solutions to a game reported before and after playing the STEM game. Children were also tested to measure if they were able to make analogical connections between the items presented in the game and the tools used to solve the real-world STEM task. With exposure to the STEM game, children may be able to perform better on the real-world task and have higher feelings and interests toward STEM.

Research Questions

In order to understand children's feelings and interest toward STEM, the current study aimed to answer several research questions: Does an increase in liking the STEM game correlate to greater understanding of the iPad game? Does more previous STEM exposure relate to higher self-efficacy in the STEM task? Does STEM exposure relate to solving the STEM task? Does more exposure to STEM affect boys' and girls' interests toward STEM activities?

METHODS

Participants

Participants were 79 children between ages 3.61 to 7.21 years ($M = 5.50$, $SD = 0.79$) with 51.9% boys (40.1% girls), and 49.4% White (30.4% Multi-Ethnic, 13.9% Hispanic/Latino, 3.8% Asian, and 2.5% Black/African American). The majority of participants (14% in lab) were recruited and interviewed in schools in the Riverside County.

Materials

In the STEM Task, children were asked to solve a problem that required them to get a ball elevated on a table into a bowl on the floor using a variety of tools (i.e., golf club, lacrosse stick, ramp, paper construction ramp, and a large spoon). The task is presented twice to measure any differences in STEM interest and self-efficacy in the pre-post difference score. Children played 2 STEM games on an iPad. The

first game was *Quack's Apples*, in which children roll an apple into pond using incline planes. The second game was *Memory Lane* in which participants recall and select items shown on and disappearing from the screen.

Measures

The participants were interviewed for 15 to 20 minutes. The interview contained 9 measures: STEM Exposure, STEM Interest (Pretest and Posttest), STEM Task (Pretest and Posttest), STEM Self-Efficacy (Pretest and Posttest), iPad Game Understanding and iPad Game Enjoyment.

STEM Exposure. STEM Exposure was measured by asking children 3 questions about their prior exposure to STEM (e.g., What is your most favorite toy?; What is your most favorite show?; What is your most favorite game to play on an iPad?). Responses were coded for if the activity (a) engaged the child in problem solving situations, (b) challenged the child to remembering a sequence or series, (c) involved math or engineering, (d) engaged children in using technology in a complex, cognitive manner, (e) taught science concepts, (f) encouraged the child to actively create or think about art or design, or (g) promoted logical thinking. Activities received a score of 1 for each STEM element included in the favorite activity. An activity was characterized as a STEM activity if it received a score of at least 4. Participants were coded as having STEM Exposure if they had 2 or more favorite activities characterized as STEM activities.

STEM Interest. To assess children's STEM Interest, children were asked 8 questions about their interest in learning about various STEM topics: (a) animals, (b) how a computer works, (c) how to stay healthy, (d) how to build a bike, (e) how to add numbers, (f) how a phone works, (g) building with blocks, and (h) counting. Children could respond to each question by pointing to a picture of a face with a Frown (-1), Neutral (0), or Smile (+1). Ratings for the 8 activities were added to determine the participant's total score, which could range from -8 to 8. Each participant had two STEM Interest scores: before game play and after game play.

STEM Task. Children attempt to solve a task of getting a ball elevated on a table into a bowl using a spoon, racetrack

ramp, lacrosse stick, paper ramp, or golf club. To solve the task, children are given one attempt in the first trial. After exposure to the iPad game, children are given three attempts in the second trial. Participants either succeed (1) by using the racetrack ramp, or fail by using any other tools (0).

STEM Self-Efficacy. After attempting to solve the STEM Task problem, children were asked to indicate (a) “How good were you at getting the ball into the bowl?” and (b) “How much did you like getting the ball into the bowl?” Children were given three response options: *not at all* (0), *a little* (1), or *a lot* (2). Each participant had two STEM Self-Efficacy scores: before game play and after game play.

iPad Game Understanding. Next, participants played the iPad game *Quack’s Apples* which served to help participants create an analogy between the iPad game and the STEM task. To measure understanding of the game’s concept, children answered “What did you use to get the apples into the pond?”

iPad Game Enjoyment. After playing the iPad games, children were asked “How much did you like helping Quack get the apples into the pond?” They were given three response options: *not at all* (0), *a little* (1), or *a lot* (2).

PROCEDURE

First, children were interviewed about their exposure to STEM. Following the interview, participants completed the STEM Interest Pretest questions, and then were presented with the STEM task of getting a ball into a bowl. At pretest, the children were only allowed one attempt to solve the problem. After this attempt, children answered the STEM Self-Efficacy Pretest questions. Then, children played *Quack’s Apples* followed by *Memory Lane*. *Memory Lane* served as a distraction so that children may be tested for their understanding of how to play and solve *Quack’s Apples*. Participants then played the STEM task again and were given three trials to complete the task correctly. Afterwards, they were reassessed for their self-efficacy towards the STEM task post exposure to more STEM from *Quack’s Apples*. Their interest in the 8 STEM questions was measured again for any significant differences after the iPad game play.

RESULTS

Does an increase in liking the STEM game correlate to greater understanding of the iPad game?

Pearson’s Bivariate Correlations examined the relation between liking the STEM game and a greater understanding of the iPad game. There was a positive correlation between liking the STEM game and a greater understanding of the iPad game ($r = 0.111$, $p = 0.292$), but no significant correlation was found. Children’s enjoyment of the game did not affect their understanding of the iPad game.

Pearson’s Bivariate Correlations examined the relation between the participants’ Self Efficacy Pretest Total vs. iPad Game Understand score average ($r = 0.02$, $p = 0.84$) and the difference was taken from the participants’ Self Efficacy Posttest Total vs. iPad Game Understanding score average ($r = 0.14$, $p = 0.23$). This shows there was a positive correlation between children’s self-efficacy and their understanding of the iPad game post game play; however, no significant correlation was found.

Does more previous STEM exposure relate to higher self-efficacy in the STEM task?

Pearson’s Bivariate Correlations examined the relation between previous STEM Exposure and Self Efficacy Pretest Total. There was a negative correlation between STEM Exposure and Self Efficacy Pretest Total ($r = -0.07$, $p = 0.52$), but no significant correlation was found. Children’s previous STEM exposure had no effect on their self-efficacy after attempting and possibly solving the STEM task in the pre-test.

Pearson’s Bivariate Correlations examined the relation between STEM Exposure and Self Efficacy Posttest Total. There was a negative correlation between STEM Exposure and Self Efficacy Posttest Total ($r = -0.1$, $p = -0.38$) but no significant correlation was found. Children’s previous STEM exposure had no effect on their self-efficacy after attempting and possibly solving the STEM task in the post-test.

Does STEM Exposure relate to solving the STEM Task?

Pearson’s Bivariate Correlations examined the relation between STEM Exposure and Solving STEM Task. There was a negative correlation between STEM Exposure and Solving STEM task ($r = -0.17$, $p = 0.13$) but no significant difference was found. Children’s previous STEM exposure

had no effect on their ability to solve the STEM task.

Independent samples *t*-tests were run to observe any differences between children's STEM Exposure, Self Efficacy Pretest, Self Efficacy Posttest, STEM Interest Pretest, and STEM Interest Posttest.

Does more exposure to STEM affect boys' and girls' interest toward certain STEM activities

An Independent Samples *t*-test was conducted to compare STEM Exposure for boys ($M = 2.71$, $SD = 0.60$) and girls ($M = 2.74$, $SD = 0.50$). The *t*-test did not display a significant difference, $t(77) = 0.236$, $p = 0.814$, Cohen's $d = 0.054$; there were no differences between boys' and girls' STEM exposure.

Another Independent Samples *t*-test compared Self Efficacy Pretest for boys ($M = 2.37$, $SD = 0.73$) and girls ($M = 2.05$, $SD = 0.66$). The *t*-test indicated a significant difference $t(77) = 1.996$, $p = 0.05$, Cohen's $d = 0.460$; boys had higher pretest self-efficacy than girls.

A further Independent Samples *t*-test compared Self Efficacy Posttest for boys ($M = 2.42$, $SD = 0.59$) and girls ($M = 2.19$, $SD = 0.93$). There was not a significant difference, $t(77) = 1.329$, $p = 0.49$, Cohen's $d = 0.295$, $M = -0.268$ for boys and $M = -0.790$ for girls. Also, an Independent Samples *t*-test indicated there was no significant increase in children's self-efficacy after the playing the STEM task, $t(77) = 0.438$, $p = 0.663$, Cohen's $d = 1.208$ $M = 0.049$ for boys, and $M = 0.1316$ for girls.

A third Independent Samples *t*-test compared STEM Interest Pretest for boys ($M = 5.17$, $SD = 2.74$) and girls ($M = 5.26$, $SD = 2.72$). The *t*-test did not show a significant difference, $t(77) = 1.50$, $p = 0.881$, Cohen's $d = 0.032$.

The last Independent Samples *t*-test compared STEM Interest Posttest for boys ($M = 4.90$, $SD = 3.34$) and girls ($M = 4.47$, $SD = 3.67$). The *t*-test did not indicate a significant difference, $t(77) = 0.544$, $p = 0.59$, Cohen's $d = 0.123$. The *t*-test Pretest Posttest difference score demonstrated there was no significant decrease in boys' and girls' interest in STEM after playing the STEM task, $t(77) = 0.942$ $p = 0.349$, Cohen's $d = 0.214$, $M = -0.268$ for boys, and $M = -0.790$ for girls.

A Paired Samples *t*-test examined if there were any differences between children's scores in the Self Efficacy & Interest Pretest vs. Posttest. The results for the Self Efficacy measure were not significantly different at pretest and posttest, $t(77) = 0.943$, $p = 0.348$, Cohen's $d = 0.108$. Children's pretest ($M = 2.22$, $SD = 0.71$) and posttest ($M = 2.30$, $SD = 0.77$) showed no significant increase in self-efficacy.

A Paired-Samples *t*-test revealed a trend toward a significant difference in children's STEM Interest from pretest to posttest, $t(77) = 1.880$, $p = 0.064$, Cohen's $d = 0.167$. Children's STEM Interest decreased slightly from pretest ($M = 5.22$, $SD = 2.71$) to posttest ($M = 4.70$, $SD = 3.48$).

DISCUSSION

This research attempted to understand the differences in children's self-efficacy and interest in STEM through their previous exposure and an increase in exposure using iPad games. The hypothesis was that an increase in liking the STEM game would correlate to greater understanding of the iPad game; more previous STEM exposure would relate to higher self-efficacy in the STEM task; previous STEM exposure would relate to solving the STEM task; and more previous exposure to STEM would correlate with children's interest toward certain STEM activities or children's self-efficacy toward a STEM task.

Most participants were unable to solve the task in the STEM Task Pretest and Posttest, but more were able to solve the STEM task during the Posttest than the Pretest. This supports that the task was too advanced for children. Some participants even found *Quack's Apples* to be difficult, which could potentially inhibit them from making analogical connections between the iPad game and the STEM task.

There was no significant correlation between STEM Exposure and Self Efficacy Pretest Total ($r = -0.07$, $p = 0.52$). And this negative trend continued in the Self Efficacy Posttest Total ($r = -0.1$, $p = -0.38$). Previous research has shown that with more exposure to STEM content, children will be able to have a higher understanding (Richert et al., 2011), but in the current research, this neither translates to a higher self-efficacy nor generates positive feelings

towards STEM. This is consistent with Meluso et al., 2012, which found that children's exposure to STEM would significantly influence their feelings toward STEM. Because most participants could not solve the STEM task, this could suggest an inhibition of learning new STEM content and therefore a lack of genuine exposure for children.

There was a negative correlation between STEM Exposure and Solving STEM task ($r = -0.17, p = 0.13$). Participants with greater previous exposure were more likely to solve the task than those who had less exposure. Again, because the STEM task was difficult, children may not have been able to demonstrate that they can understand and apply the analogical concept from *Quack's Apples* to the STEM task. Thus, previous exposure to STEM would not be sufficient for children's performance in solving the STEM task.

Like Miller et al. (2011), the experimental design did

not foster significant increase in children's interest in STEM. However, many did enjoy the STEM game, which supports Gros (2007), in which the interactivity of games may foster children's interest and feelings in STEM. It is possible that because children do not solve the STEM task, they may not feel confident in their abilities or that they are currently performing well. This could potentially lead to a negative mood and an immediate decrease in their STEM interests. This does not suggest that children overall will have a permanent decrease in STEM. Giving children positive reinforcement may be an important factor to consider when teaching children STEM concepts. In the future, expanding the population surveyed and tailoring the STEM task and STEM iPad game towards the children's appropriate age level may yield improvement in STEM interest and feelings. Changing the study to be longitudinal could allow future research to have a deeper understanding of children's self-efficacy and interests.

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This research could not have been conducted without the support of the Chancellor's Research Fellowship, and especially Dr. Richert's guidance and mentorship and the Childhood Cognition Lab. Camp Apple Tree, Bethel Christian School, First United Methodist Preschool, and First Baptist Preschool have also been very supportive of the project by providing their location and encouraging their students to participate in the research study.

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Determination of Immune Signal–Receptors, PD-1 and PD-L1, Interactions in Solution using qFRET Technology

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ABSTRACT

Human PD-L1 (programmed cell death 1 - ligand 1) is a transmembrane protein that is highly expressed on the membrane of cancer cells. It binds to inhibitory receptor PD-1 (programmed cell death protein - 1), which is expressed on the surface of cytotoxic T cells. The interaction between PD-L1 and PD-1 reduces the effect of anti-tumor immune response and the strategy of blocking their interaction has been used for anti-cancer drug manufacture. Past studies isolated the extracellular domain of PD-L1 for characterization of the structure. This study aims to recover, isolate, and purify the insoluble PD-L1 protein (external domain), and study its binding interaction with PD-1 for the development of an *in vitro* quantitative FRET (qFRET) assay. To report PD-L1/PD-1 binding interaction, fluorescent donor and acceptor pairs, CyPet and YPet were bound to PD-L1 and PD-1 proteins respectively and qFRET was applied to assess the interaction of the two proteins based entirely on fluorescence.

The results provide evidence of recovery of the purified and refolded CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins from cell pellets, shown in the coomassie stained SDS-PAGE gel. This study also shows that the proteins were able to be recovered through affinity chromatography under denaturing conditions. The qFRET technique showed that the acceptor, YPet-Ext.PD1, is interacting with the donor, CyPet-Ext.PDL1. This study provides a novel method for better understanding the binding mechanism of PD-L1/PD-1 that can be applied to other cell-surface protein interactions, as well as to stipulate a platform for small molecule inhibitor related drug screenings and production.

Keywords: PD-1, PD-L1, Expression, Immunotherapy, Antibody, FRET



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Mary Nwangwu is a fourth-year bioengineering major. She joined the Liao Lab in June 2017 and is presently working on protein renaturing and Förster resonance energy transfer-based high throughput screening assays. Mary's research on fluorescent tagged protein derivation from inclusion bodies is being funded by the Undergraduate Education Quarterly Minigrant. She is also currently working on a senior design project and intends to pursue a career in biotechnology.



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INTRODUCTION

Cancer cells are characterized as cells that divide persistently and out of control. These abnormal cells form solid malignant tumors (abnormal body tissue growth) or flood the bloodstream. Cell division is a standard process in the body for tissue repair and growth. Healthy cells will divide until it is no longer necessary for them to do so, but cancerous cells continue to produce more copies of themselves. The classification of a cancer cell depends on the cell type from which it originates. These categories include, but are not limited to, Carcinoma (originating in membranous tissues that line the body), Sarcoma (originating in connective tissue such as muscle and bone), and Leukaemia (originating in tissues that produce new blood cells, mainly bone marrow).

Cancer immunotherapy involves the use of the immune system to treat cancer. The immune system consists of two parts: innate immunity and adaptive immunity. Innate immunity, or in built immune protection, is viewed as the defense an individual has from birth. The defenses rely heavily on barriers such as the skin, inner linings of organs, hair, inflammatory responses, and natural killer cells. Adaptive, or acquired, immunity is characterized as the protection developed after exposure to certain bacteria or diseases. The defenses include antibody production (B cells) and potentiating the function of accessory cells (natural killer cells, macrophages, etc.). Cell mediated response (T cells) is also a part of the adaptive immunity branch and is especially useful for fighting cancer cells. Different chemicals that can aid in the immune response can be produced *in vitro*. These different types of immunotherapy include monoclonal antibodies (mABs), vaccines, cytokines, and adoptive cell transfer.

Human PD-L1 (programmed cell death-ligand 1) is a transmembrane protein that is expressed on a wide variety of normal tissues, including natural killer cells, B cells, and endothelial cells.⁹ It normally binds to inhibitory PD-1 (programmed cell death protein-1) receptors expressed on the surface of activated cytotoxic T cells. Cytotoxic T cells, or T lymphocytes, are central effectors to eliminate cancer cells in an antigen and cell contact dependent manner and induce long-lasting tumor regression.⁸ PD-1/PD-L1 interaction inhibits T cell growth and the secretion

of cytokines, polypeptides that act on hematopoietic stem cells (cells that give rise to myeloid and lymphoid blood cells) and modulates immune and inflammatory responses. Hino et al. found that tumor cell-borne PD-L1 induces the apoptosis (programmed cell death) of tumor-specific T cell clones *in vitro*, suggesting the potential role of PD-L1 in tumor immunity.⁶

PD-L1 expression is heterogeneous across primary breast cancers and is generally associated with the presence of tumor-infiltrating lymphocytes and the presence of poor-prognosis features such as high grade, and aggressive molecular subtypes (triple-negative (TN), basal, HER2-enriched).² Clinical findings have shown that blockage of PD-1 and PD-L1 interaction by antibodies has a steadfast effect on many advanced tumors (*see Figure 1*). Monoclonal antibodies are a class of drugs called checkpoint inhibitors that hinder the interaction of PD-1 and PD-L1 and overcome the disadvantages of conventional anti-cancer therapy. *In vitro* and *in vivo* studies that were done by Lussier et al. showed that blocking PD-1 using an antibody can partially enhance T-cell function.¹

Sales of the PD-1/PD-L1 therapy class have grown from \$84 million in 2014 to \$6,292 million in 2016, with five PD-1/PD-L1 inhibitors presently approved across a variety of tumor indications.⁵ However, the developers of successful PD-1/PD-L1 based immunotherapies such as OPDIVO® and KEYTRUDA® still continue to face clinical and commercial challenges. As discussed by Meng et al., these challenges include the identification of optimal combinations, treatment-related adverse effects, the high cost and lack of effective predictive markers which make the aforementioned products less promising as widely used therapeutic options.¹¹ The utilization of qFRET to assess the small-scale binding mechanism of PD-1/PD-L1 can provide kinetic measurements that will give insight into what kind of effects these drugs will have overall. The overarching goal is to find a drug that will have optimal efficacy and minimal side effects.

The FRET (Förster resonance energy transfer) phenomenon is used to identify protein interactions, monitor intracellular signaling activities in real-time, and survey bioactive molecules by high-throughput screening.¹³ It is a distance-

dependent physical process by which energy is transferred nonradiatively from an excited molecular fluorophore (the donor) to another fluorophore (the acceptor) by means of intermolecular long-range dipole-dipole coupling.¹² The excitation of the donor can elicit an energy transfer to induce emission from the acceptor when the two are close to each other (1-10 nm). This results in quenching of the donor and excitation of the acceptor. YPet (yellow fluorescent protein for energy transfer) is the acceptor fluorophore and CyPet (cyan fluorescent protein for energy transfer) is the donor fluorophore. Protein-protein interactions allow for CyPet and YPet to come into close range, permitting FRET to occur successfully. The quantitation of FRET can be made with a ratio metric determination of the two signals, as FRET results in both a decrease in fluorescence of the donor molecule as well as an increase in fluorescence of the acceptor.⁷

This study plans to recover, isolate, and purify both CyPet-Ext.PDL1 and YPet-Ext.PD-1 proteins. The efficiency of this procedure will be verified by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). A fluorescence spectrum of CyPet-Ext.PDL1 and YPet-Ext.PD1 will be generated to confirm the presence of the fluorescent tags. The qFRET technique will then be applied to the two proteins and will provide evidence of binding interaction between PD-L1 and PD-1.

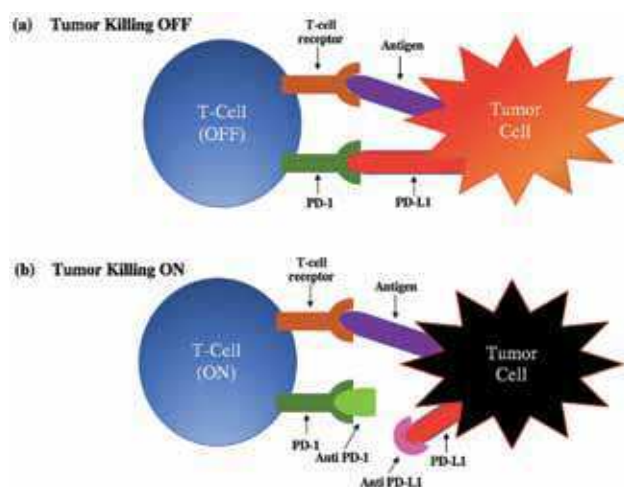


Figure 1: Schematic of immune checkpoint mechanisms. (a) Tumors can express PD-L1, which interacts with PD-1 receptors found on T cells, leading to suppression of the anti-tumor T cell response. (b) Inhibitors for PD-L1 and PD-1 prevent this interaction, unleashing the T cell anti-tumor response.

2. MATERIALS AND METHODS

2.1 Cloning and expression of CyPet-Ext.PDL1 and YPet-Ext.PD1

The recombinant vectors pET28(b)-CyPet-Ext.PDL1 and pET28(b)-YPet-Ext.PD1 (external domain: extracellular domain plus transmembrane) were cloned into competent *Escherichia coli* DH5 α bacterial cells and respectively plated on LB agar plates supplemented with 50 μ g/ml kanamycin. The recombinant vectors were then transferred to *Escherichia coli* BL21(DE3) for expression of recombinant protein. Transformed *E. coli* BL21(DE3) were plated on LB plates supplemented with 50 μ g/ml kanamycin to ensure transformation.⁹ Single isolated clones of CyPet-Ext.PDL1 and YPet-Ext.PD1 were inoculated in 10 mL LB tubes and incubated overnight at 37°C with 220 rpm (revolutions per minute). A 1 L culture was then inoculated with 10 mL of the starting culture and allowed to grow to exponential phase approximately for 3 hours at 37°C and 220 rpm with shaking, Isopropyl β -D-1-thiogalactopyranoside (IPTG) in final concentration of 0.2 mM was added to induce, or enhance the expression of, the proteins. Aliquots were collected before and after addition of IPTG for SDS-PAGE analysis. The incubation parameters were lowered to 20°C and 150 rpm with shaking and the culture allowed to express for 16 hours.

2.2 Purification of recombinant CyPet-Ext.PDL1 and YPet-Ext.PD1

CyPet-Ext.PDL1 and YPet-Ext.PD1 cells were harvested by centrifugation for 15 minutes and pellets were washed and resuspended in lysis buffer then subjected to sonication for 15 minutes for each sample. After sonication cell lysates were centrifuged at 35,000 g for 30 minutes, and both supernatants and pellets were examined by using 10% SDS-PAGE to verify the location and expression of CyPet-Ext.PDL1 and YPet-Ext.PD1. The pellets were resuspended in denaturing buffer, incubated at 37°C for 15 minutes, and further incubated overnight at 4°C and shaken to completely dissolve the pellets and denature the proteins within the solution. Finally, the denatured proteins were centrifuged and the supernatants, which held the dissolved and denatured proteins, were purified by affinity chromatography on nickel nitrilotriacetic acid (Ni-NTA) gel matrix. Columns of Ni-NTA nitrocellulose resin were washed and equilibrated with denaturing buffer

first, then cleared cell lysates (containing CyPet-Ext.PDL1 and YPet-Ext.PD1) were loaded onto separate columns to be rinsed with wash buffer. The fractions were collected and examined with SDS-PAGE to evaluate the purity. The supernatants were also subjected to a Bradford assay to examine the concentrations of the CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins.

2.3 Refolding of recombinant CyPet-Ext.PDL1 and YPet-Ext.PD1

The proteins must be dialyzed against dialysis buffer in order to remove the imidazole concentration and wash buffer. Protein filtrates for CyPet-Ext.PDL1 and YPet-Ext.PD1 were obtained via column chromatography and

Buffer/Solution	Reagents	pH
Lysis Buffer	20 M Tris-HCL, 0.5 M NaCl, 5 mM Imidazole	7.4
Wash Buffer and Denaturing Buffer	6M Guanidine-HCL, 0.5M Arg, 50 mM Tris-HCL, 0.5 M NaCl, 10 mM DTT	8.0
Dialysis Buffer A	4 M Guanidine-HCL, 0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer B	2 M Guanidine-HCL, 0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer C	1 M Guanidine-HCL, 0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer D	0.5 M Guanidine-HCL, 0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer E	0.25 M Guanidine-HCL, 0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer F	0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer G	0.25 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0
Dialysis Buffer H	0.5 M Arg, 50 mM Tris-HCL, 50 mM NaCl, 1 M DTT	8.0

Table 1
List of different buffers used in expression and purification of protein

poured into economical biotech dialysis membranes and dialyzed against dialysis buffer containing decreasing concentrations of Guanidine-HCL (6M, 4M, 2M, 1M, 0.5M, 0.25M and 0M – dialysis buffers A through H) for 16 hours in each concentration (*see Table 1*).

2.4 qFRET assay

30 μL of purified and refolded recombinant CyPet-Ext.PDL1 and YPet-Ext.PD1, both at 0.5 μM , were transferred into a 384-well plate (Greiner black) and the fluorescence emission spectrum of each well was measured with a fluorescence multi-well plate reader (Molecular Devices, FlexstationII³⁸⁴). Recombinant CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins were also mixed together and diluted with phosphate buffered saline (PBS) to a total volume of 30 μL . The final concentration of CyPet-Ext.PDL1 was fixed to 0.5 μM and the final concentration of YPet-Ext.PD1 varied from 0 to 6 μM . The mixtures were also transferred into a 384-well plate and the fluorescence emission spectrum of each well was measured. Two excitation wavelengths were used: 414 nm to excite CyPet, and 475 nm to excite YPet (*see Figure 2*).

The concentration of YPet-Ext.PD1 in both free and bound forms can be converted to functions of E_{MFRET} (sensitized emission from YPet-Ext.PD1), since it is proportional to the amount of YPet-Ext.PD1 bound to CyPet-Ext.PDL1. Song et al. derived an equation for E_{MFRET} , where FL_{DD} is the excited fluorescence signal of donor, FL_{AA} is the excited fluorescence signal of acceptor, 'x' is the CyPet ratio factor, and 'y' is the YPet ratio factor (*see Equation 1*).¹³

$$E_{\text{MFRET}} = (E_{\text{Mtotal}}) - (x * FL_{\text{dd}}) - (y * FL_{\text{AA}}) \quad (1)$$

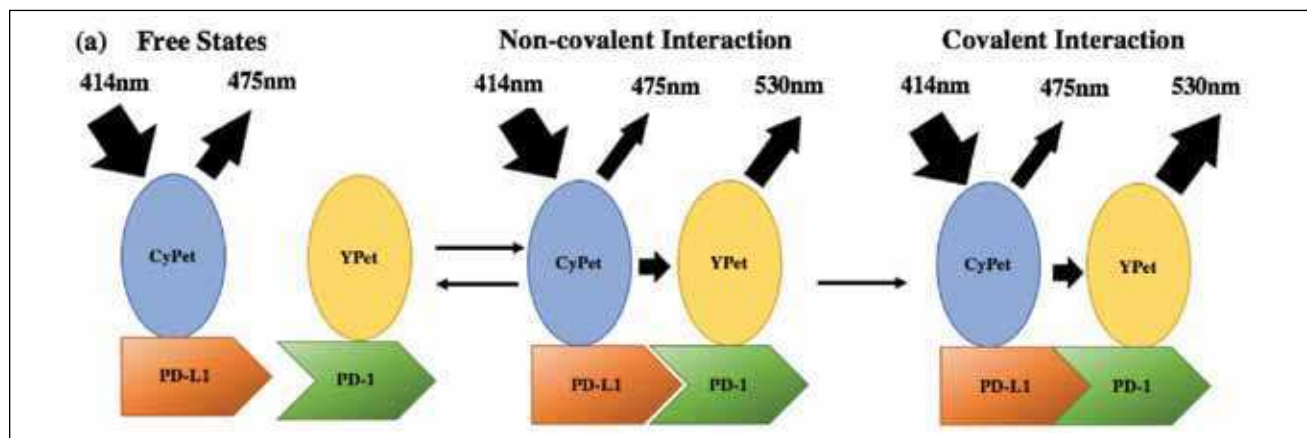


Figure 2: Design and detection of high sensitive FRET-based detection for PD-L1/PD-1 protein interactions.

Protein	Volume Cultured	IPTG Concentration	Fluorescent Protein Yield	% Purity based on SDS-PAGE
CyPet-Ext.PDL1	2 L	0.2 mM	0.2 μ M	~80
YPet-Ext.PD1	1 L	0.2 mM	8 μ M	~87

Table 2

3. Results

3.1 Expression, purification, and dialysis of recombinant proteins

Induced, un-induced, cell pellet, cell lysate, and purified & refolded (from inclusion bodies) samples for both YPet-Ext.PD1 and CyPet-Ext.PDL1 were examined by using 10% SDS-PAGE to detect the active expression products (see Figure 3a). The induced and un-induced samples for both YPet-Ext.PD1 and CyPet-Ext.PDL1 (Figure 3a – lanes 1, 2, 6 & 7) show thicker bands than the others, denoting some protein expression, with the induced sample displaying higher expression.

Cell pellet and cell lysate samples for both YPet-Ext.PD1 and CyPet-Ext.PDL1 (Figure 3a – lanes 3, 4, 8 & 9) were also examined. The cell pellet samples for both proteins displayed darker and thicker bands than their cell lysate correlates. The concentration results via Bradford assay of the supernatants (lysates) and pellets for CyPet-Ext.PDL1 and YPet-Ext.PD1 showed high concentration of Ext.

PDL1 protein but low concentration of Ext.PD1 protein (see Table 2). The purified, dialyzed, and refolded samples of YPet-Ext.PD1 and CyPet-Ext.PDL1, rescued from their cell pellets, were visualized in Figure 3a – lanes 5 & 10. These bands were lighter than the bands shown for their cell pellets (Figure 3a – lanes 3 & 8), correlating to the recovery and purification of the proteins of interest from pellets and cellular debris.

3.2 qFRET confirmation

Measuring the fluorescence spectrum of both purified and refolded CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins showed a maximum RFU (relative fluorescent unit) value for CyPet-Ext.PDL1 at approximately 3.8×10^5 for its emission wavelength of 475 nm and a maximum RFU value for YPet-Ext.PD1 at approximately 1.6×10^7 for its emission wavelength of 530 nm (Figure 3b). It was found that the CyPet fluorescent protein has a lower quantum yield than the YPet protein, thus emission at the same protein concentration was lower for CyPet-Ext.PDL1.⁴ The E_{MFRET} versus concentration of YPet-Ext.PD1 plot shows an increase in E_{MFRET} as the concentration of the acceptor (YPet-Ext.PD1) increases (see Figure 4).

4. DISCUSSION

4.1 PD-L1/PD-1

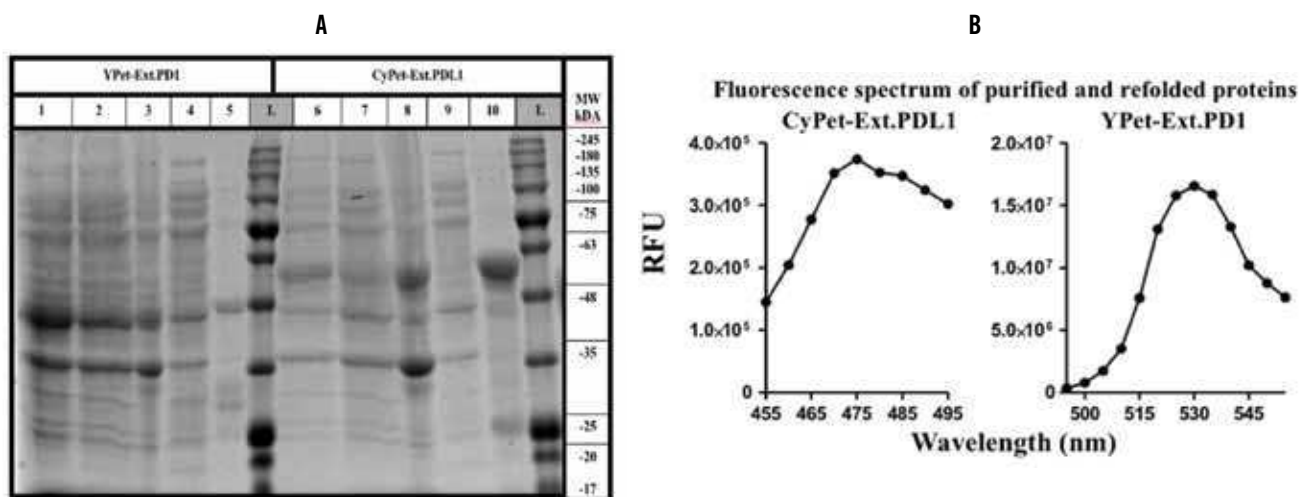


Figure 3: (a) SDS-PAGE gel, coomassie stain for determination of protein expression and validation of protein purification. 1-BL21(DE3) YPet-Ext.PD1 Un-induced, 2-BL21(DE3) YPet-Ext.PD1 Induced expression, 3-Cell Pellet, 4-Cell Lysate, 5-Purified and refolded from inclusion body YPet-Ext.PD1, 6-BL21(DE3) CyPet-Ext.PDL1 Un-induced, 7-BL21(DE3) CyPet-Ext.PDL1 Induced expression, 8-Cell Pellet, 9-Cell Lysate, 10-Purified and refolded from inclusion body CyPet-Ext.PDL1. MW of YPet-Ext.PD1 is 48 kDa and CyPet-Ext.PDL1 is 58 kDa. (b) The fluorescence spectrum was measured on FlexstationII384 of CyPet-Ext.PDL1 and YPet-Ext.PD1 both at $0.5 \mu\text{M}$, with excitation at 414 nm and 475 nm respectively. The CyPet fluorescent protein has a lower quantum yield than the YPet protein, thus emission at the same concentration is lower.

Expression of the induced and un-induced CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins (**Figure 3a – lanes 1, 2, 6 & 7**) demonstrated that the pET28(b) vector functioned properly. It was observed that the samples of the cell pellets for both CyPet-Ext.PDL1 and YPet-Ext.PD1 held higher amounts of protein than in their cell lysates (**Figure 3a – lanes 3, 4, 8 & 9**), correlating to the understanding that PD-1 and PD-L1 are insoluble proteins that need to be rescued from cell pellets. **Figure 3a – lanes 5 & 10** confirmed the ability to recover, isolate, and purify these proteins out of the denaturing conditions. CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins were able to remain solubilized once the denaturant (guanidine) had been removed from the dialysis buffers, and the addition of arginine to the buffers (**shown in Table 1**) helped the proteins stay in solution. It was observed in previous iterations that without arginine, the proteins of interest would go back to being insoluble after removal of the denaturant.¹⁴

4.2 qFRET

The fluorescence spectrum of CyPet-Ext.PDL1 and YPet-Ext.PD1 shown in **Figure 3b** provided evidence of the presence of isolated fluorescent tagged proteins and confirmed that the CyPet fluorescent protein has a lower quantum yield than the YPet fluorescent protein. The E_{mFRET} plot in **Figure 4** showed that as the concentration of YPet-Ext.PD1 increased from 0 μM to 1.0 μM , and the CyPet-Ext.PDL1 concentration was held constant at 0.25 μM , there was an increase in E_{mFRET} RFU. This provided evidence of acceptor (YPet-Ext.PD1) and donor (CyPet-

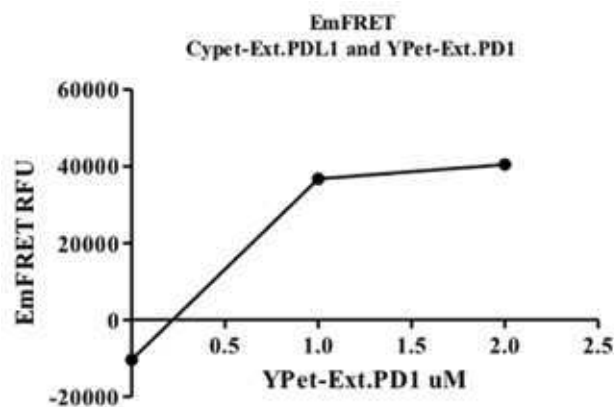


Figure 4: Plot of E_{mFRET} versus the acceptor YPet-Ext.PD1. The donor, CyPet-Ext.PDL1, concentration was held constant at 0.25 μM and the concentration of YPet-Ext.PD1 was increased. The readings were taken on the FlexStationII384. Plot generated on GraphPad Prism.

Ext.PDL1) interaction. Furthermore, as YPet-Ext.PD1 concentration increased from 1.0 μM to 2.0 μM , a maximal FRET signal, or a plateau in the FRET signal, was noted. This implied that all of the CyPet-Ext.PDL1 and YPet-Ext.PD1 were paired and no additional FRET signal could be detected.

5. Conclusion

PD-L1 has particular importance as a potential target for adaptive immunity and the protein-protein interaction of PD-L1 and PD-1 is a highly valued drug target for cancer immunotherapy. Past studies isolated the extracellular domain of PD-L1 for characterization of the structure. In this study, the insolubilized CyPet-Ext.PDL1 and YPet-Ext.PD1 proteins were put through a dialysis array to solubilize them. After completely removing the denaturant, protein precipitation was overcome by adding arginine, correlating to previous studies.¹⁴ The initial yields of YPet-Ext.PD1 and CyPet-Ext.PDL1 (denatured within the cell lysate) were 22 μM and 0.5 μM respectively. The final yields were observed to be 8 μM for purified YPet-Ext.PD1 and 0.2 μM for purified CyPet-Ext.PDL1, showing that a large amount of protein was lost during the dialysis process. Additionally, a large difference between the inherent expression levels of PD-1 and PD-L1 was observed. The fluorescence spectrum measurement verified independent fluorescent tagged protein activity and the qFRET technique applied to the combination of YPet-Ext.PD1 and CyPet-Ext.PDL1 proteins reported their binding interaction.

Currently, methods to improve the initial yield of CyPet-Ext.PDL1 through codon optimization and or a SUMO tag to enhance expression levels are being investigated.^{3,10} For future studies, a protein kinetic analysis of the K_d (dissociation constant) between PD-1 and PD-L1 can be performed. The results acquired in this experiment give insight into a novel technology, qFRET, that can be utilized to study the interaction between PD-1 and PD-L1. This technique is a platform for future research into high-throughput drug screening of small molecule inhibitors including antibodies, protein kinetic studies, and the utilization of FRET as a reporter for cell-surface protein interactions. Quantitative FRET technology holds a bright future for discovering immunotherapeutic agents with high efficacy and tolerability.

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The Queer Confessional: Foregrounding the Discordant Poetics of Henri Cole Through the Troubling of Genre

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ABSTRACT

This paper is a research study on a nascent American Poet, Henri Cole, whose scholarly archive remains minimal, positioning itself as a fundamental starting point to which to begin a discussion around a new, but critical voice within the field of American Poetics. The discordant poetics of Henri Cole are informed by two distinct traditions within the canon of 20th and 21st century American poetics, namely that of a queer and confessional mode to which he stands at the intersection of both movements. However, Cole's scholarly archive is woefully small due to his recent publication presence in the literary field. Thus, research was focused on analyzing Cole's chief poetic inspirations (Hart Crane and Elizabeth Bishop) as well as recursive forays into queerness and confessional poetics. In identifying that intersection, I argue that Cole is at the forefront of troubling poetic genre through the proposal of a 'queer confessional' mode of poetics. Cole troubles the notion of truth through utilizing a queer perspective on the confessional genre that has historically fell to criticisms of histrionics and overt inwardness. Instead, this paper aims to subvert a long history of literary criticism through instead focusing on how a queer confessional form 'retrieves' the confessional as a radical, poetic gesture that relishes in the instability of truth-making through a simultaneous re-affirmation of poetic genre. Cole thus disrupts and revises the notion of literary tradition through his queer perspective; in which his 'queer confessional' proposes a poetics of liberation.

KEY WORDS: Henri Cole, Queerness, Queer theory, Poetics, Apollonian-Dionysian, Confessional



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INTRODUCTION

*“all I am is impulse and longing / Pulled forward by the rope of your arm” – Henri Cole, *Blur**

To contextualize Henri Cole’s nascent place within the often changing canon of contemporary American poetry, I turn to Sasha Weiss’ interview of the poet published in the *Paris Review* to begin to illuminate Cole’s purpose as a poet. Cole remarks “Pleasure comes from the art-making impulse, from assembling language into art.” This paper will examine Cole’s conception of pleasure, both sexual and aesthetic, as is configured in the poetics of Henri Cole. In thinking about pleasure, it is critical to note that Cole himself is in conversation with a queer tradition of American Poetics in which he is inspired by Hart Crane and Elizabeth Bishop. His poetic mission is rooted in finding that queer pleasure in language assembly by way of the queer configuration of his lived experiences. However, this question of queerness finds a further complication in the question of what type of poetry, or genre, that Cole engages in conversation with. This paper will draw from a field of American Poetics called ‘confessional’ poetics that is more personally-driven. However, applying the genre of confessional poetics proves difficult in Cole’s reticence to the term ‘confessional.’ He defines it as, quoted from the same *Paris Review* interview, “more diary-like and confined to the here and now and without much aesthetic dignity.” His reticence troubles a simplistic foregrounding and thus, this is where I turn to the larger goal of this paper. I aim to chart a new terminology and existence of poetics, more specifically the notion of queer confession, that places Cole’s verse at the forefront of the troubling of poetic genre.

Through an analysis of Henri Cole’s discordant poetics, this paper charts the existence of a new kind of confessional poetics that resists the notion of what Miranda Sherwin identifies as “associated with private, self-revelatory impulses that are insufficiently and transparently transformed into art” (15). These ‘private, self-revelatory impulses’ play a key part in foregrounding how confessional poetics is perceived in American literary scholarship. However, reducing confessional poetics to a self-centered modality does not account for the political

and queer possibilities of confessional poetry. Thus, I argue for an existence of a queer confessional mode of poetics through a close analysis of Cole’s verse that manifests as an acceptance of contradictions and an existence of in-betweenness. Critical in reading confessional poetics is to take stock of an autobiographical lens that considers the poet’s life experiences in sync with their written verse. However, a queer confessional model de-stabilizes an over-reliance on autobiographical ‘truth.’ Queer confession espouses a ‘truth’ that exists in constant conflict with itself and doubts its own existence via contradiction. The acceptance of an unstable truth from unstable life is in turn an acceptance of both ‘self-love’ and ‘self-hate,’ a queer configuration that is articulated by a confessional mode.

AN APOLLONIAN-DIONYSIAN FRAMEWORK

To begin to identify how the queer confessional manifests in Cole’s poetics is to first articulate the Apollonian-Dionysian literary dynamic. The Apollonian-Dionysian literary framework is a model that allows for Cole to engage in a poetics of liberation that informs the queer confessional. From a literary viewpoint, the Apollonian-Dionysian framework operates as a juxtaposition of seemingly opposing forces similar to a binary. The Apollonian is presented as an orderly schema while the Dionysian pulses with discordant language. Cole’s poetry deliberately blurs the line between both. He utilizes Apollo’s order and Dionysus’ disorder in tandem to create a ‘framed disorder.’ This ‘framed disorder’ is precisely the queer function that Cole engages by troubling two concepts that appear to be, at the surface, opposites and instead engages and mixes both through deliberate contradiction. “Apollo,” The conclusory poem of *The Visible Man*, published in 1998, is exemplary of the framework of the ‘framed disorder’ that I am proposing. Divided in sequences of 14, the second ‘part’ of Apollo is what exemplifies Cole’s poetic framework of a dual ‘order’ and ‘chaos’ in his verse:

*Stay married, god said. One marriage.
Don’t abortion. Ugly mortal sin.
Beautiful Gorgeous Mary loves you
so much. Heaven tremendous thrill
of ecstasy forever. What you are,
they once was, God said, the beloved ones
before you... (1-7)*

These quoted lines are ‘Apollonian’ through the terseness of its construction. Performing a line-by-line reading, the sentences in this poem are almost fragmented and are consistently so throughout. The Apollonian order is inscribed through the pattern of terseness that is maintained in the first two lines. As one moves further forward, elements of the Dionysian ‘disorder’ begin to manifest through the lengthening of each sentence. The brevity of God’s entreaty away from sin ‘expands’ in length at the invocation of Mary. However, if the order of length is disrupted, the language seems to remain Apollonian in length and construction. Yet the growth of each line is gradual and remains compact. The almost unyielding pattern belies a discordant Dionysian impulse. “Heaven tremendous thrill of ecstasy forever” is one such illusory line that appears to be a carefully constructed in an orderly, Apollonian fashion. Its Dionysian, disorderly pulses are embedded in its ‘tremendous thrill of ecstasy,’ invoking the image of Dionysian chaos that is formed through an Apollonian assemblage.

The Apollonian-Dionysian framework is crucial to articulating the queer confessional framework and in identifying Cole’s configuration of queerness. Langdon Hammer makes a salient observation about the dual existence of the Apollonian and Dionysian dynamic by writing of his “desire to combine in his work the qualities of formal balance and open-ended, anarchic exploration that have longed defined opposing or even warring principles in American poetry.” Hammer’s lucid points elucidate the introductory analysis of “Apollo” through the remarking of a ‘formal balance,’ a formulation defined by faithfully hewing to traditional poetic form, that Cole utilizes to maintain the tightly knit Apollonian order of his poetry. However, this does not mean that the Dionysian, ‘open-ended anarchic exploration’ is at war with Cole’s ‘formal balance.’ Instead, Cole accepts the contradiction of a ‘framed disorder,’ utilizing the Apollonian and Dionysian in tandem. In the 10th sequence of Apollo, Cole is cogent in the realization of ‘assembling the words,’ in which he writes “Yet, subject is / only pretext for assembling the words / whose real story is process is flow” (3-5). Picking up on the notion of ‘language assembly,’ the ‘subject’ is Cole’s Apollonian moment of laying the framework of

‘assembling the words.’ However, Hammer’s invocation of Dionysian “free-for-all sensuality’ is what creates the ‘process’ of the ‘real story.’ For Cole’s poetry, order and disorder are intimately connected in ways that reading them as opposed to each other in a binary is unproductive. Thus, what is at stake is Cole’s relishing of complication and instability in his poetic writing in which the Apollonian-Dionysian literary framework is taken as a step-by-step process. Apollonian order is the first step of the “pretext” that finds its rough ‘conclusion’ in flow. However, Dionysian disorder is by no means the conclusion to the process of ‘flow.’ I return to Hammer’s salient remarks on the Apollonian-Dionysian literary dynamic, to which he writes “We can see Cole’s Apollonian Dionysian impulses, ‘mixed up’ like those two counterpointed voices.” The mixing of what seems to be two ends of a binary is instead the flowing of contradictions into one another. Thus, it is through the identification of these Apollonian-Dionysian contradictions is where we begin to unpack the particular queerness of Cole’s verse.

UNSETTLING THE CONFSSIONAL

After unpacking the Apollonian-Dionysian dynamic in Cole’s verse, I turn to analyzing the implications of confessional poetics’ legacy as a juncture in Henri Cole’s expression of queerness. Of course, Cole remarks somewhat negatively on the notion of his poetry being classified in the confessional vein, “When I am writing, there is no pleasure in revealing the facts of my life. Pleasure comes from the art-making impulse, from assembling language into art.” It would be unproductive to impose the label of the confessional onto Cole. He acknowledges the term’s existence but does not classify himself as such. However, what I propose is not necessarily imposing the confessional genre as we know it towards Cole’s verse. As this paper proposes a new model of looking towards confessional poetics via the queer confessional, the possibility of re-imagining the confessional is rooted in a critical intersection between private, ‘self-insulated’ spaces and its political ramifications. At stake in the intersection of public/private is also the crossing between poetic confession and Cole’s queerness. Cole’s verse is in conversation with such intersections. Accordingly, his poetry is deeply invested in an examination of private,

family life (especially with a focus on the mother figure) that is also present in confessional poetics. A salient example would be “Mechanical Soft” from Cole’s *Touch*:

...Mother is dying,
you see, and proximity to this death makes me
nostalgic for the French language. I am not
a typical son, I suppose, valuing happiness,
even while spooning mechanically soft pears—
like light vanishing—into the body whose tissue
once dissolved to create breast milk for me (8-14)

The intensely personal nature of “Mechanical Soft” is indeed almost confessional-like in the traditional sense of the term. The speaker engages with the mother’s active death and expresses the intensely personal complications of the emotional turmoil of the mother’s death. The chief focus on the mother figure is another marker in which Cole displays a deep familiarity with the conventional tropes of confessional poetry. It must also be acknowledged that just as Cole utilizes its tropes, he engages in its subversions as well. In contrast to a confessional poem’s clear, photo-like language to its verse, Cole’s confessional verse is Apollonian in language and sparse in detail. The language is fantastical and surreal, the ‘mechanically soft pears’ rendering a much more unstable image in which ‘spooning’ these fantasy fruits is rooted in the pleasure of language assembly amid the mourning of the mother’s death.

I then turn towards Peter Nickowitz’s *Rhetoric and Sexuality* in further tracing the legacy of confessional poetics towards Cole’s work. Nickowitz’s research delves into a deep analysis of Cole’s chief poetic inspirations, in which Elizabeth Bishop configures as my introductory junction point through her relationship of confessional poetics as Nickowitz writes that she “distrusted the confessional movement.” Despite such a relationship, two prongs “betrays a certain tolerance for it,” namely her relationship to Robert Lowell (a major figure in 20th century confessional poetry) and her reliance upon ‘truth.’ This distrust finds an arguable continuation in Cole’s self-perception in his *Paris Review* interview. Cole makes a similar gesture, as the interviewer remarks “You’ve said you see yourself not as a confessional poet, but as an autobiographical poet.”

However, it is important to note that the term ‘confessional’ is in constant flux due to a long history of literary criticism that often derides the term. Thus, to negotiate such criticisms, this paper will turn towards analyzing the nature of “truth” and myth-making as constellatory points in which the term ‘confessional’ moves away from the impulses of an absolute truth and more into the radical function of de-stabilizing the notion of what a ‘truth’ is. Through exploring Bishop’s relationship with the truth, which Nickowitz writes as a “reliance on ‘truthfulness’ functions as one way that the poet asserts a perspective,” I argue that Bishop’s ‘truth’ is not absolute insofar that it is not required for the truth to be rooted to an absolute truth. Instead, I motion for a ‘truth’ that is unstable in poetic verse. There is an element that is ‘confessed,’ and thus, there is an element and reliance of the truth. Yet, truth itself is unstable. It is in this instability in which the confessional can exist as a radical and queer space. Thus, this de-stabilization of an absolute truth is the ‘retrieval’ of the confessional mode and the foundational marker of its intersections towards a queer mode.

QUEER CONFSSION IN PRACTICE

A look through Cole’s expression of queerness is necessary to articulate the queer confessional in practice. Indeed, the locus point of Cole’s queerness is located within the ‘framed disorder’ of his verse, of which his troubling of the Apollonian and Dionysian (order and disorder) binary is at the root of his poetic queerness. However, the queer function of the Apollonian-Dionysian literary framework only scratches the surface of how Cole troubles the notion of poetic genre and form. To further complicate this poetic queerness, the ‘confessions’ that Cole expresses in his poems are tied to two critical aspects of queer theory in conversation with poetry, mother-eroticism and the poetics of difficulty. Mother-eroticism, which Nickowitz identifies, is “desire for the mother functions as a basis for homoerotic desire” (54) and the poetics of difficulty is an expression of lived difficulty that scholar Robert K. Martin identifies as being an integral aspect to Hart Crane’s lived, queer experience. With these two aspects, Cole thus engages with the necessary queerness that troubles not only the notion of ‘truth,’ but perhaps troubles the notion of pure, literary confession which necessarily requires a clear ‘truth’ in verse. The maternal figure, with Nickowitz’s identification

of mother-eroticism in mind, is a salient conduit to which to engage and identify queer confession in practice. The maternal is located in the poem “Touch:”

*Then I lay down beside you,
dissolving loneliness,
and the white maggots wriggled*

*As the preacher spoke,
no one seemed to hear him,
tamping their eyes, touching one another. (24-29)*

The troubling of truth is performed in these six lines, through which Cole lies down and even physically desires to be with the mother figure represented through the ‘you.’ The sustained Apollonian sparseness of Cole’s verse is a deliberate troubling of truth that destabilizes the confessional-esque language of the surrealist action of Cole’s speaker lying near the dead mother’s body. Indeed, the images present in the verse are ones that take a simultaneous pleasure and refusal to elucidate a clear truth. The carefully constructed verse renders truth in Dionysian instability. The notion of truth is presented as a slippage through which the surrealism of Cole’s ‘lying down’ with the mother figure is not meant to be taken as a literal. Instead, Cole invites the reader to question precisely what is being confessed through his sparse writing. The feeling of grief is the primary emotion that Cole’s speaker emphasizes as the confessional moment of maternal loss. There are no ‘white maggots’ that physically wriggle as Cole’s speaker imagines himself and his own body to lie down next to his mother’s grave, but the image of the maggot serves as the facilitation of Cole’s mood of profound maternal loss. Cole’s bodily actions through intimate interaction with the mother and maternal grief intersect to trouble and queer the confessional form, through which this very moment is queer confession.

The questioning of truth is fundamental for a queer poet such as Cole, whose marginalized existence is reflected upon the loss of the maternal. In the framework of mother-eroticism, the loss of the mother marks a profound loss for a queer, male subject; through which even the expression of loss becomes rooted in a difficulty to which even expression proves nigh-impossible. Hart Crane, a queer, early 20th century American poet and one of Cole’s chief poetic

inspirations, engages in these similar thematics of maternal grief and is a salient example of queer theory’s poetics of difficulty in a far more precarious and homophobic time. Robert K. Martin identifies Crane’s dilemma and difficulty, as the “dilemma was double, since for him the plight of the homosexual in a heterosexual society and the plight of the artist in a materialistic were conjoined” (117). Martin then motions towards a solution with two prongs, in which sexual and political anxiety must be resolved to begin to negotiate a queer existence. I motion that the nature of Cole’s inspiration of Crane isn’t to necessarily offer a solution but is instead the acknowledgement of poetic difficulty and to articulate the struggle of being a queer poet. Martin identifies the ‘plight of the homosexual in heterosexual society,’ and that plight does indeed exist today with the current body politic being ambiguous and antagonistic towards queer folk. Thus, perhaps finding a solution to queer struggle isn’t necessarily what’s at stake. Instead, the tools to articulate queer struggle and sexuality is at the core of the relationship between Cole and Crane. Through that close examination, examining the poetics of difficulty through Cole’s literary predecessors becomes integral into investigating how his poetics operate in a queer American framework.

A CONCLUSORY NOTE: ARTICULATING A QUEER CONFSSIONAL

The three angles of the Apollonian-Dionysian literary framework, identifying and unpacking the confessional form in the context of Cole’s verse, and then putting queer confession in practice is a prototypical articulation of the queer confessional model. However, I stress the slippery and nascent nature of this model. The notion of queer confession is foregrounded upon conceptual paradigms (queerness and confession) that are themselves considered unstable and do not have a fixed definition. Thus, defining the term must be attentive to the implications of slippage. Queer confession must relish in instability, especially when the notions of truth and life experiences are called into question, becoming deliberately clouded in poetic verse. This foregrounding has only taken a brief foray into each of these three angles. However, further study requires a more holistic look in Cole’s bibliography as well as a more in-depth look into queer and confessional literature. In doing so, perhaps we can further the project of re-defining

what confessional poetics means in our present moment and its inseparability from queerness and the body politic. A queer perspective on literary tradition proves fruitful in ‘retrieving’ and ‘liberating’ the confessional tradition towards a poetics of liberation. The inseparability from the body politic is necessary in foregrounding the political work of queer confession, of which turning towards the

personal and private space carries within it a statement of defiance, embodiment, and a refusal of heteronormative subjectivity. The queer confessional is a proposal of poetic possibility, liberating tradition and deliberately engaging with difficulty to propose a new genre, and perhaps even a new modality, of poetics in our present moment.

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Protocol for Mice Behavioral Analysis in Response to Predator Cues

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ABSTRACT

Mice exhibit defensive behaviors in response to various predator cues. When a mouse “senses” a predator at a close distance, it exhibits freezing behavior. Alternatively, when it senses bodily excretions from a predator, it escapes from the area. These behaviors are evolutionary responses to predators that help their increase survival. How animals sense the different types of predator-derived cues and induce appropriate behaviors in response to the specific predator cues have largely remained elusive.

In this study, we aimed to establish a method to analyze mouse behavioral responses toward various forms of predator-derived biological samples, such as cat saliva, which contain chemical cues. We categorized mouse responses to predator cue exposure as freezing, fear assessment, or exploratory behavior, each of which is triggered by different levels of fear that the animal is experiencing. The behaviors were quantified manually and compared between the animals exposed to control and predator-cue stimuli. We show that this protocol is effective in analyzing levels of fear in mice as there is a significant increase in the occurrence of fear-based behaviors in mice exposed to cat saliva.

Developing a strong protocol for quantifying fear-related behaviors is essential to understand brain mechanisms underlying behavioral responses induced by different types of predator cues in mice. Moreover, the present protocol can be further utilized to understand how different levels of fear are processed in an animal’s brain circuitry.



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Dr. Sachiko Haga-Yamanaka is an Assistant Professor in the Department of Molecular, Cell and Systems Biology. Dr. Haga-Yamanaka received her PhD from the University of Tokyo. Dr. Haga-Yamanaka has focused on the mouse vomeronasal system that detects and discriminates specific molecular cues provided as pheromones and predator cues. These chemical cues trigger a repertoire of innate behaviors, including mating and defensive behaviors. Interestingly, brain neurons in the vomeronasal circuitry express a variety of hormone receptors, suggesting a potential mechanistic link between the vomeronasal circuitry and endocrine hormones. Using an *in vitro* cell culture system and *in vivo* mouse models, she aims to elucidate how the brain controls behavior through integration of external sensory signals and internal hormonal state.



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Sunaina Santhiveeran is a fourth year Neuroscience major. Her research with Dr. Sachiko Haga-Yamanaka focuses on creating behavioral protocols to be used to uncover the mechanisms behind the defensive behavior exhibited by mice exposed to predator samples. Other notable experiences throughout her undergraduate career include working with large data sets as a UCR FIELDS intern at Caltech/JPL and volunteering in various capacities in the healthcare field. Her future plans are to combine these past experiences and pursue graduate studies in Translational Medicine in order to help increase access to quality healthcare through technology. Her work with Dr. Sachiko Haga-Yamanaka is supported by the University Honors Program and the Undergraduate Research Mini-Grant.

INTRODUCTION

Animals make behavioral decisions in response to external stimuli. For example, most prey animals have the ability to perceive the presence of a predator and exhibit defensive behaviors to increase the likelihood of their survival. When a prey animal “senses” a predator at close distance, it exhibits freezing behavior in order to disappear from the predator’s vision. Freezing behavior is defined by a lack of movement for several seconds, allowing prey to disappear from the predator’s view. Alternatively, when prey senses bodily excretions from a predator, it escapes from the area. This fleeing behavior is exhibited when the predator is in the area. These behaviors are defensive behaviors, practiced to escape predator detection or capture. Laboratory mice exhibit these defensive behaviors and other behavioral responses, such as paying attention solely to the predator cue while maintaining movement, approaching the cue in a hesitant manner, hiding in bedding, and examining the cue by extending their head and neck towards it while keeping their body away. These are stereotypic behavioral responses of prey animals practiced in order to maximize the chance of survival (Blanchard et al. 2001).

Prey rodents such as mice are able to perceive the presence of a predator by sensing chemical cues emitted from predators (Apfelbach et al. 2005; Osada et al. 2015.), which are detected by the main and accessory olfactory systems (Papes et al., 2010; Dewan et. al., 2013.). Interestingly, even inbred rodents, which have been isolated in the laboratory from other species for hundreds of generations, are known to respond to predator-derived cues and exhibit fear-like defensive behaviors to chemical cues contained in the saliva, urine, and feces of predators (Apfelbach et al. 2015.). This innate response suggests that the neural mechanism underlying this behavioral decision is genetically determined.

In order to understand this genetically-determined neural mechanism, in this study, we aimed to establish a standard method to analyze mouse behavior responses towards various forms of predator-derived biological samples containing chemical cues. For this purpose, we established three critical components of the analysis: a behavioral assay system, behavioral analysis platform, and mouse defensive behavior categorization. In the post hoc analysis, mouse

behavior responses were categorized based on types of behaviors, types of exploratory sniffing, direction facing, and relative locations of the mouse.

By using this method, we found a significant increase in fear-related behavior responses, such as freezing, when mice were exposed to certain predator samples as compared to controls, indicating this method can be used for future behavioral analyses of predator defensive behavior in mice. Developing a strong protocol for quantifying fear-related defensive behavior is essential for understanding the neural mechanisms underlying the behavioral responses induced by different types of predator cues. Moreover, the present protocol can be further utilized to understand how different levels of fear are processed in an animal’s brain circuitry.

METHODS

Habituation

Mice were habituated for three days before the recorded trial. Habituation was conducted by transporting the subject’s home cage to the recording room and keeping them there for an hour after the experimenter leaves.

Behavior Assay

The recorded trial was conducted at the same time as the habituations. Before transporting the mice to the recording room, the food and water bottle were removed and the lids were replaced with flat lids. The bedding was also removed to eliminate all visual obstructions. Upon transportation into the recording room, the mice were habituated for an hour. The experimenter entered the room after the hour and placed the sample after ten minutes of allowing the mice to habituate to their presence. Once the sample was placed, the experimenter remained in the room for the duration of the video recording.

Samples were collected from domestic cats usually within 24 hours of each trial. These samples were either fur clippings or saliva swabs. A control was also conducted by placing a plain cotton swab in the home cage. The forceps were clean before each use.

Recording the Mice Videos

Videos were recorded using infrared lights and a night vision camera during the dark cycle. The videos were

Behavior	Description
Interacting	Mouse touches/plays with the stimulus. At the minimum, this is observed with the mouse prodding the sample with their nose and can also include the mouse actively playing with the sample. With cotton swabs, this often means the mouse bites it or chews it. With the fur samples, mice typically move it around or hold it.
Attending	Mouse stays still with head facing towards the stimulus, directing all attention to it. This can be differentiated from freezing behavior because there is still movement with the mouse, often actively sniffing towards the sample or moving towards or away from the sample. Typically, attending behavior is most often observed during the time of sample placement. This class of behavior is not frequently observed as it is an intermediate between freezing and searching + object sniffing.
Approaching	Mouse heads in a straight line towards the stimulus. This behavior is often accompanied with stretched sniffing. Mice often take roundabout routes along the walls of their enclosure or other ways to avoid the sample when they first approach it. Approaching behavior differentiates these more fearful approaches with a direct, exploratory approach. This is an exploratory behavior.
Hiding	Mouse burrows self in bedding. Not all mice tested were housed with enough bedding to burrow themselves in so this behavior was rare. This is an indication of fear.
Freezing	Mouse is still or shows minimal movement. Exceptions are respiration and movements associated with sniffing that last less than roughly a one second. This is the highest indication of fear.
Immobile	Mouse is still due to freezing or sleeping. This category exists for instances when the mouse's behavior is inconclusive, normally due to them facing away from the camera.
Sleeping	Mouse is still, with their body relatively curled up, their eyes closed, exhibiting respiratory movements as well as occasional twitching. This is a relaxed behavior and normally indicates a low level of fear.
Digging	Mouse digs through the bedding in the enclosure. This is an exploratory behavior and normally indicates a low level of fear.
Grooming	Mouse licks and cleans itself. This is a relaxed behavior and normally indicates a low level of fear.
Eating	Mouse eats, nibbles, or chews on something. This behavior category is not used if the object being gnawed is the sample; that would be under "interacting." Mice aren't provided any food during recording so it's often bedding or excrement that is consumed. This behavior category is rare but indicates a low level of fear as it is a relaxed behavior.
Rearing	Mouse stands on their back legs. This is typically an exploratory behavior and normally indicates a low level of fear. When exploratory, the mouse faces upwards towards the top of the enclosure and often grasps on the walls or the ceiling grid of the enclosure. Rearing can also be accompanied by freezing behavior when the mouse freezes in an upright position.
Searching	This is a default behavior. It typically involves the mouse wandering their environment but is used anytime the mouse is not performing any of the other defined behaviors. This is an exploratory behavior and normally indicates a low level of fear.
Undirected sniffing	This is a default behavior. It typically involves the mouse wandering their environment but is used anytime the mouse is not performing any of the other defined sniffing behaviors. This is an exploratory behavior and normally indicates a low level of fear.
Stretched sniffing	Mouse sniffs and stretches forward at the neck, holding their body back, in order to get closer to the sample. This is a fear assessment behavior and normally indicates a moderate level of fear.
Object sniffing	Mouse sniffs the object at close proximity. If the mouse stretches to sniff the object better, the behavior is then coded as "stretched sniffing." This particular form of object sniffing involves the mouse being close enough to touch the object. This form of object sniffing always accompanies the "interacting" behavior. This is a fear assessment or exploratory behavior and normally indicates a low to moderate level of fear.
Object sniffing (far)	Mouse sniffs towards the object from a distance in this form of object sniffing. This particular form is a fear assessment behavior and normally indicates a moderate to high level of fear.
Middle	Mouse is in the center of the enclosure with no part of their body (except for the tail) touching the walls. This category helps determine location and is based on the fact that samples were typically placed away from the walls of the enclosure. This is either a fear assessment or exploratory behavior.
Corners	Mouse is along the walls of the enclosure with some sort of physical contact with the walls (except for the tail). This category helps determine location and relates to higher level of fears since fearful mice were observed to hug the walls when navigating the enclosure or freezing during fear assessment or exploratory behaviors.
Towards sample	Used to better describe any of the previous behaviors. Mouse is facing the sample. When in combination with searching, typically means the mouse is moving in the direction of the sample. Cannot be used alone to determine level of fear.
Away from sample	Used to better describe any of the previous behaviors. Mouse is facing away from the sample. When in combination with searching, typically means the mouse is moving away from the sample. Cannot be used alone to determine level of fear.

Table 1. Description of the behaviors coded into BORIS.

recorded for roughly five minutes before sample placement and fifty-five minutes after placement.

Behavior Categorization

Table 1 details the categories used to quantify behaviors. These categories were determined upon watching the reactions of mice when exposed to cat saliva and based on the ethogram presented by Dr. Joseph Garner’s lab at the Stanford School of Medicine (Garner et. al.). The behaviors were then organized into subcategories for fear behaviors, fear assessment behaviors, exploratory behaviors, location, and relaxed behaviors, based on the subcategories outlined by Blanchard et. al. (Blanchard et. al., 2003).

Fear behaviors include freezing and hiding. Freezing was determined to be a complete lack of movement, excluding sniffing movements that lasted less than two seconds. Hiding was only observed when mice had bedding to burrow in. Fleeing behavior was not observed in this experiment due to space constraints of the cages.

Fear assessment includes stretched sniffing, approaching, and attending. These behaviors indicate a level of caution and involve the mouse keeping their body at a distance while extending their head and neck towards the sample.

Exploratory behaviors were determined to encompass object sniffing, interacting, rearing, undirected sniffing, and searching. Undirected sniffing and searching were set to be the default behaviors. They are used to describe basic exploratory behaviors when no other specific behavior was observed. Exploratory behaviors are driven more by curiosity than fear.

Relaxed behaviors were performed when the mouse is feeling little to no fear and includes grooming, sleeping, and eating. Such behaviors are typically only performed when the mouse feels safe in their environment.

Location was observed to correlate with fear behavior when estimated as either middle or corners. Since videos were only recorded laterally, this was estimated based on

Behavior code	Excluded behaviors
freezing	hiding, approaching, interacting, searching, eating, grooming, sleeping, digging
hiding	freezing, approaching, interacting, rearing, searching, eating, grooming, sleeping, digging
stretched sniffing	undirected sniffing, object sniffing
undirected sniffing	stretched sniffing, object sniffing
object sniffing	stretched sniffing, undirected sniffing
attending	approaching, interacting, searching, eating, grooming, sleeping, digging
approaching	freezing, hiding, attending, interacting, rearing, searching, eating, grooming, sleeping, digging
interacting	freezing, hiding, attending, approaching, rearing, searching, eating, grooming, sleeping, digging
rearing	hiding, approaching, interacting, searching, eating, grooming, sleeping, digging
searching	freezing, hiding, attending, approaching, interacting, rearing, eating, grooming, sleeping, digging
middle	corners
corners	middle
eating	freezing, hiding, attending, approaching, interacting, rearing, searching, grooming, sleeping, digging
grooming	freezing, hiding, attending, approaching, interacting, rearing, searching, eating, sleeping, digging
sleeping	freezing, hiding, attending, approaching, interacting, rearing, searching, eating, grooming, digging
digging	freezing, hiding, attending, approaching, interacting, rearing, searching, eating, grooming, sleeping
towards sample	away from sample
away from sample	towards sample

Table 2. Description of the behavioral exclusions coded into BORIS.

whether or not the mouse's body (not including the tail) was touching the walls of the enclosure. Samples were usually dropped in the middle of the cage, so mice remaining in corners are likely indicators of caution or fear.

Combining these four types of behavior, general behaviors, sniffing behaviors, location, and direction facing, gives us a good idea of the level of fear the subject is experiencing. For example, a mouse exhibiting relaxed behaviors like grooming or eating in the middle of the cage exhibits low levels of fear. A mouse keeping to the corners and displaying defensive behaviors like stretched sniffing is experiencing more fear.

Behavior Coding

Coding originally began five minutes before sample placement to an hour after placement. Observations of initial trials/videos showed that most fear behavior occurred within 20 minutes after sample placement, so future coding was conducted from two minutes before sample placement to 20 minutes after sample placement. This seems to have been adequate, as fear behavior tended to decrease over time exposed. All coding was conducted using BORIS Behavioral Analysis software (Friard et. al., 2016).

BORIS is a software that allows users to define their own behaviors and then manually code them to either a live video or a prerecorded video. Behaviors can also be grouped into categories or given modifiers to better describe events. Users can also define their own independent variables as well. The program is also capable of conducting basic statistical analysis, including providing average durations for observed behaviors and the export of the data in Excel form.

This feature was utilized to omit behaviors that would be exclusive to one another, ensuring that at any given point of the coding, there is only one of each type of behavior being performed. The exclusions programmed into BORIS are featured in *Table 2*.

RESULTS

A total of thirty videos were analyzed using this protocol. The coding was analyzed by looking at the length of time each behavior was coded for over the twenty minutes that were analyzed. BORIS also provides raster plots of the

behaviors coded for each video. *Figure 1* shows a raster plot for both a control and a mouse exposed to cat saliva. These can be used to visualize the behaviors observed.

A comparison of the data collected for three controls and three trials of cat saliva exposure can be found in the bar graphs in *Figure 2a*. *Figure 2a* shows that mice exposed to the cat saliva exhibited significantly less object sniffing and significantly more undirected sniffing. Object sniffing from afar and stretched sniffing were not significantly different between the control and saliva-exposed mice. *Figure 2b* shows that there is no significant difference in the direction facing between control mice and saliva-exposed mice, though there is a tendency for control mice to face towards the sample more often than away whereas saliva-exposed mice tended to face both ways for similar amounts of time. In *Figure 2c*, while there is no significant difference in the behaviors of the control mice and saliva-exposed mice, there was a tendency for the control mice to remain in the middle of the cage longer compared to trial mice, of which tended to stay near the corners. *Figure 2d* shows significant increase in freezing behavior and a significant decrease in rearing behaviors and interacting in the cat saliva exposed mice. There is also a noticeable decrease in relaxed behaviors, including grooming and digging, that was observed in control mice.

DISCUSSION

Using this method, we found a few significant differences between the control and the saliva-exposure trials. The mice exposed to the control swabs interacted with it for far longer than the mouse with the sample swab, indicating low levels of fear. There was also a significant increase in freezing behavior expressed with the cat saliva that wasn't expressed with the control, indicating a high level of fear. This matched expectations that there would be increased fear in mice presented with predatory cues. There was a less significant difference in the direction facing behaviors between the two groups, indicating that this category may not be necessary in future analysis. Although there was a trend for control mice to stay within the middle of the cage while saliva-exposed mice stay near the corners, this difference was not significant and may need further specification. For example, an overhead recording would allow for a more specific analysis of distance from the predator sample. The location and direction facing values

could, in addition, be used in conjunction with the other behaviors to describe them better.

Overall, the method presented proved useful for analyzing behavior in response to predator cues and would be well-suited for analyzing fear responses to other stimuli as well. The results follows expectations of increased fear behavior in response to predator cues, ensuring that it is a fit method for future fear-behavior analysis.

CONCLUSION

This method provides a foundation for analysis of mouse defensive behavior responses to predator cues. The significant increase observed in fear behaviors catalogued using this method of analysis matches expectations that mice react defensively in response to cat chemical cues. This method could be further combined with neural recording and neural manipulation techniques to uncover the brain regions associated with the perception of fear. Revealing these underlying neural mechanisms is not

only significant in elucidating how sensory signals are processed to trigger behavior, but also in understanding the brain mechanisms of fear and anxiety in humans.

ACKNOWLEDGEMENTS

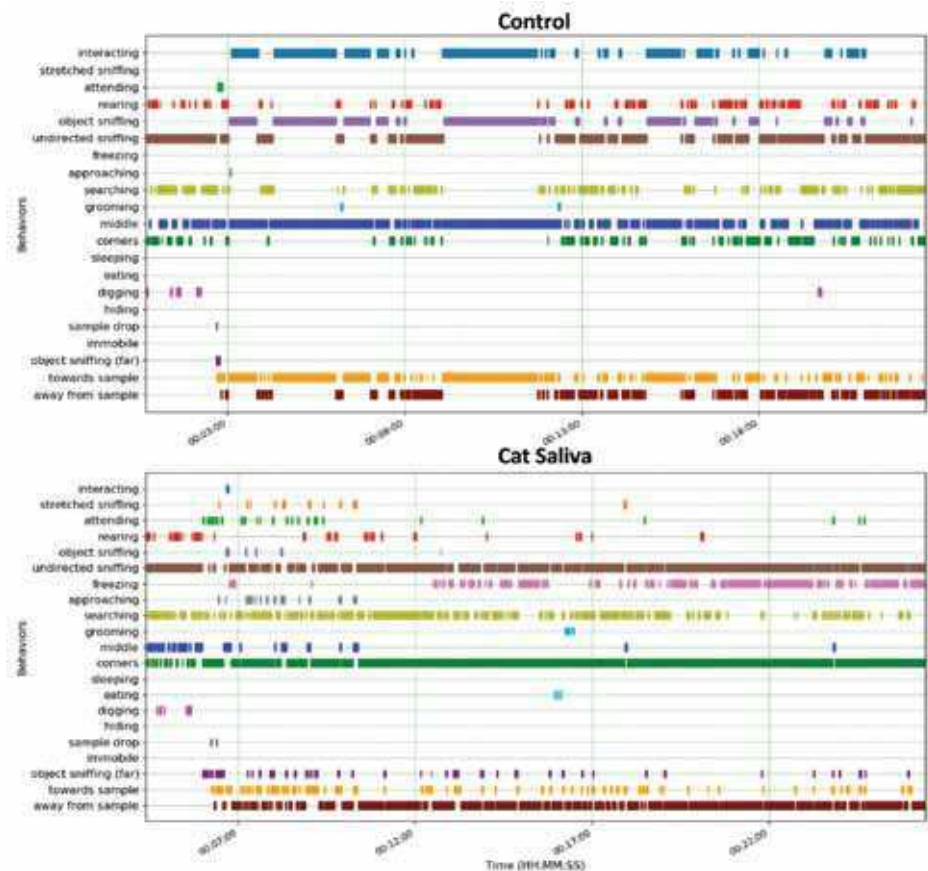
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Figure 1. Raster plots of mouse behavior in control mice (top) and cat saliva exposed mice. (bottom). Differences to note include: the decreased interaction with the saliva sample, the lack of stretched sniffing with the control, the increase in object sniffing with the control, the complete lack of freezing behavior in the control, the decreased incidence of approaching with the control, the increased time spent in corners than the middle with the saliva sample, and the increase in object sniffing (far) with the control.



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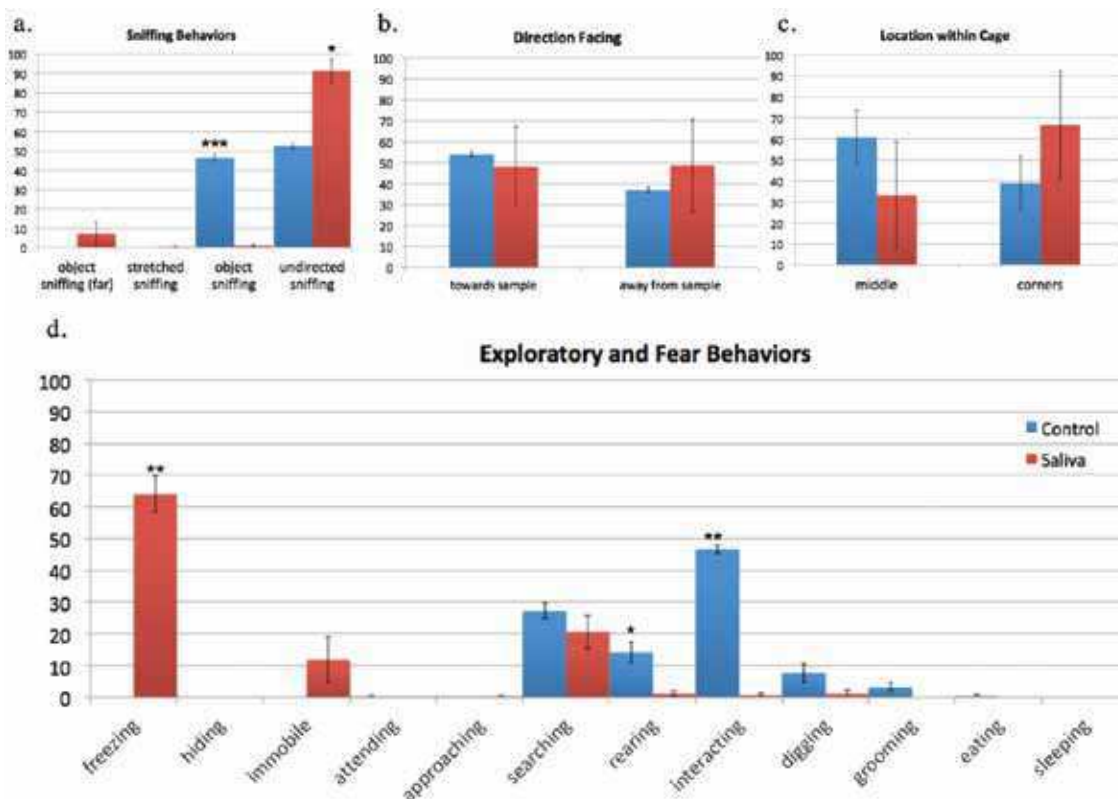


Figure 2. Bar graphs displaying percent of total time of mouse behaviors. Values are averages \pm SEM; $n = 3$ mice. Significance was determined using a two-tailed T test with 2 degrees of freedom. **Figure 2a** shows a significant difference in object sniffing and undirected sniffing. **Figure 2b** compares the incidence of the direction facing for each trial type. There is no significant difference for either but a trend can be seen with the control mice exhibiting a tendency to face towards from the sample. **Figure 2c** compares the location of mice in both trials. There is no significant difference for either but a trend can be seen with the control mice exhibiting a tendency to stay in the middle of the sample and vice versa for the saliva trial mice. **Figure 2d** shows a significant increase in freezing behavior and a significant decrease in interacting and rearing behaviors with the saliva exposed mice. There is also a non-significant but noticeable decrease in digging and grooming behaviors in the exposed mice as well.

Identification of Targetable Surfaces of Cks1 for Development of New Cancer Therapeutics

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ABSTRACT

Cyclin-dependent protein kinase regulatory subunit 1 (Cks1) is involved in cell cycle progression through interactions with cyclin-dependent kinases (CDK) and ubiquitination of cyclin-dependent kinase inhibitors (CKI). Dysfunction of CDK dependent associations can affect the entrance of a cell into mitosis, particularly the G1-S phase transition. Abnormal assistance from Cks1 with the multiprotein complex SCF (Skp2) in the ubiquitination of CDKN1B (p27^{Kip1}) can disrupt the mitotic regulatory protein levels escalating to cancer development. In this study, we use computational methods to investigate interactions in three different complexes sharing Cks1 in order to create a targeted pharmaceutical solution that will assist in regulation. Our analysis is performed using the crystal structure of Cks1 in three complexes to include involvement between one ubiquitin ligase and two CDKs. The assessment is based on the intermolecular electrostatic interactions, such as hydrogen bonds and charge-charge interactions. We observe that charge and hydrogen bonding plays a significant role in the stability between Cks1 and adjacent proteins in each complex. Due to large binding interfaces and varied distribution of charge across the main contact regions, we decided that a condensed pocket on Cks1 that interacts with phosphorylated p27^{Kip1} should be selected for further in-depth examination.



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Nolan Winicki is a third-year Bioengineering major. Nolan has been doing research in the Biomolecular Modeling and Design Lab under the guidance of Dr. Dimitrios Morikis since his sophomore year. He studies mitotic regulatory proteins for the creation of targeted cancer therapeutics and is currently a member of the Student Editorial Board for the University of California, Riverside Undergraduate Research Journal. Nolan plans to pursue a career in medicine.



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Professor Morikis' work focuses on immune system function and regulation, structure-dynamics-interactions-activity/function relations, design of peptides and proteins with tailored properties, drug and biomarker discovery, development of structural and translational bioinformatics methods, and systems immunology and disease modeling. His research is predominantly computational, with emphasis on molecular dynamics simulations, electrostatic calculations, free energy calculations, pharmacophore modeling, virtual screening, and protein-ligand docking, and has an experimental component, with emphasis on binding, biochemical, and functional assays and NMR spectroscopy. Nolan's work includes graduate student Reed Harrison, and is a collaborative project with Professor Jefferson Perry of UCR's Department of Biochemistry.

INTRODUCTION

During interphase of the cell cycle in eukaryotes, the G₁, S, and G₂ phases occur sequentially, and during the transition from G₁ to S there is a checkpoint to determine whether or not the cell will replicate cellular DNA. Once crossed, the cell will divide into two daughter cells and carry out normal cell processes until the next G₁ phase without interruption. Cyclin Dependent Kinases (CDK) form complexes with cyclins during short periods of the cell cycle to regulate steps of progression. Specifically, cyclin-dependent protein kinase regulatory subunit 1 (Cks1) binds to a catalytic subunit, a cyclin-dependent kinase. Regulation of the cell cycle depends on the interactions between cyclins, CDKs, and CDK inhibitors (CKIs). Dysregulation of the cell cycle results in uncontrolled, damaged cell multiplication that may lead to cancerous growths.

While the concentration of mitotic regulatory proteins oscillates during stages of the cell cycle due to periodic proteolysis, the ubiquitin-proteasome system maintains the levels of degradation.⁶ This system involves multiple enzymes and a ubiquitin ligase which dictates the specificity of ubiquitination. One of the ligases is the S-phase kinase-associated protein 2 (Skp2) which is part of the SCFSkp2 complex. This complex targets cell cycle control elements, primarily p27 and p21, tagging them for destruction.^{4,5} Cyclin-dependent kinase inhibitor 1B (p27^{Kip1}) is incredibly influential on cell cycle division due to binding and preventing the activation of E-CDK2 during the G₁ phase.³ While levels of p27^{Kip1} fluctuate with the cell cycle, modulation is done by the SCF-Skp2 complex creating an inverse relationship of concentrations.⁴ While normal levels regulate cell cycle progression, a recent study showed that overexpression of Skp2 is frequently observed in human cancer progression.² In addition, Skp2 inactivation restricts cancer development when observed in oncogenic conditions *in vivo*.¹ Cks1 plays a critical role in p27^{Kip1} ubiquitination by increasing the binding affinity of Skp2 for p27^{Kip1}.

Using structural analyses of protein complexes that focus on hydrogen bonds and electrostatic interactions, this project will search for the amino acid interactions between proteins that contribute the largest amount of stability to the protein complex. Determining key interactions can help design

new drugs to alter protein-protein interactions. These drugs have potential applications as new cancer therapeutics, acting by restoring regulation of cell cycle progress and slowing the growth rate of tumors. Additionally, the shape of protein-protein interfaces are considered to account for druggability of protein-protein interactions. After determining the most promising interactions, molecular dynamics simulations will be performed for fine-grain analysis leading to identification of key amino acid interactions that a new therapeutic should disrupt.

METHODS

Structure Retrieval and Preparation

The crystallographic structures for protein complexes Skp1-Ckp2-Cks1-p27^{Kip1}, CDK1-Cks1, CDK2-Cks1 were retrieved from the Protein Databank (PDB) with identifiers 2AST, 4YC6, and 1BUH, respectively, and were visualized using the software UCSF Chimera.¹¹ The initial structures were missing hydrogens and heavy atoms in addition to a chimeric region on Skp2 which however was not involved in the binding interface. These discrepancies were remedied through the tools PDBFixer and Modeller, and the hydrogens were assigned based at pH 7.4.

Structural Analysis of Hydrogen Bonding

Four separate analyses were performed to characterize the structural and electrostatic components of interactions in the protein complexes. The first test was to search for hydrogen donor-acceptor pairs to determine potential attractive dipole-dipole interactions that could add to the stability of the complex. This was done by quantitatively measuring the amount of hydrogen bonds and the distance between each heavy atoms. The default hydrogen bond constraints in UCSF Chimera¹⁰ with relaxations of 0.4 Å and 30° were applied to ensure that all bond distances fell within the normal 3.5Å range and had acceptable angles of bonding.

Computational Alanine Scans

Next, we performed a computational alanine scan to assess the contributions of charged sidechains to the stabilities of the protein complexes. To conduct this assessment, we used the alanine scan method from the computational framework AESOP (Analysis Of Electrostatic Structures of Proteins)¹²⁻¹⁵. This scan determined the overall contribution in terms of energy (kJ/mol) of each ionizable amino acid

when mutated to alanine individually and compared to the parent protein resulting in a change of energy ($\Delta\Delta G$). Each of the energy fluctuations out of the normal range for thermal fluctuations (± 2.5 kJ/mol) were considered to be potentially influential residues that stabilize the protein complex.

Structural Comparisons of Electrostatic Surfaces

In order to compare electrostatic complementarity at the interface of each protein complex, we calculated electrostatic potentials for Cdk1, Cdk2, Cks1, Skp2 and p27^{Kip1}. The grid of electrostatic potentials calculated by the Adaptive Poisson-Boltzmann Solver (APBS)¹⁸ were projected onto the surface of each individual protein structure within UCSF Chimera. The contacts were shown through Chimera, utilizing the find contacts and clashes feature then highlighting the selected residues to be reflected on the surface map. PBD2PQR was used to parameterize atomic radii and charges.^{16,17} Surface potentials were visualized by coloring regions according to their electrostatic potential. The color scale ranges from red at -6 kT/e, the potential energy for a single proton in the corresponding region, to white at 0 kT/e and from white at 0 kT/e to blue at 6 kT/e. At this point, the complexes were then separated and displayed in the open book conformation to qualitatively assess the possible electrostatic complementarity at the contacting surfaces.

Molecular Dynamics

Finally, molecular dynamics analysis was performed

on the complex of p27^{Kip1} and Cks1 using NAMD and the CHARM27 force field. The structures were initially minimized with the water molecules contained in the crystallographic structure and subsequently solvated in a cubic TIP3P water box measuring 61.57 Å x 63.62 Å x 53.46 Å. Sodium and chloride ions were also added to the water box, bringing the ionic strength to 150 mM. Minimization of the solvated structure was performed for 25,000 steps (50 ps) before heating to 310 K over 64 ps. Next, equilibration was performed where all protein atoms were constrained at 10 kcal/mol/Å² initially and then relaxed to 5, 2, and 1 kcal/mol/Å² before removing constraints altogether for the final equilibration run. Following equilibration, the MD simulation was run with periodic boundary conditions, Langevin temperature and pressure control, and particle mesh Ewald electrostatics. Relevant parameters for the simulation included a nonbonded interaction cutoff of 12 Å and a switching distance of 10 Å. Hydrogen bonds were held constant according to the SHAKE algorithm as an integration time step of 2 fs was used. The simulation was run over a 100ns timescale benchmarked at 50ps timestamps.

RESULTS

Intermolecular Hydrogen Bonds

The intermolecular hydrogen bonds between Cks1 and the adjacent chains in each of the three complexes are displayed (*Tables 1-3*) with the protein, three letter amino acid code, sequence number, and atom type identified for

Donor				Distance (Å)	Acceptor			
Protein	Residue	Seq. #	Atom		Protein	Residue	Seq. #	Atom
Skp2	ARG	398	NH2	3.38	Cks1	LEU	37	O
Cks1	SER	41	OG	2.77	Skp2	ASP	319	OD2
Cks1	ARG	20	NH1	3.19	p27 ^{Kip1}	THR	187	OG1
Cks1	THR	35	OG1	2.94	Skp2	THR	400	O
p27 ^{Kip1}	LYS	190	NZ	3.08	Cks1	TYR	8	O

Table 1 details the intermolecular hydrogen bond interactions in complex Skp2-Cks1-p27^{Kip1} between two amino acids (donor and acceptor) of differing proteins at the complex interface, a total of 5 hydrogen bonds.

Donor				Distance (Å)	Acceptor			
Protein	Residue	Seq. #	Atom		Protein	Residue	Seq. #	Atom
Cdk1	HIS	205	N	2.73	Cks1	GLU	63	OE2
Cdk1	GLU	206	N	3.12	Cks1	GLU	63	OE1
Cdk1	ASN	239	N	2.78	Cks1	ASP	14	OD1

Table 2 details the intermolecular hydrogen bond interactions in complex Cdk1-Cks1 between two amino acids (donor and acceptor) of differing proteins at the complex interface, a total of 3 hydrogen bonds.

Donor				Distance (Å)	Acceptor			
Protein	Residue	Seq. #	Atom		Protein	Residue	Seq. #	Atom
Cdk2	GLU	208	N	3.13	Cks1	GLU	63	OE1
Cdk2	GLU	208	N	2.85	Cks1	GLU	63	OE2
Cdk2	ILE	209	N	2.92	Cks1	GLU	63	OE1
Cdk2	LYS	237	NZ	3.05	Cks1	ILE	59	O
Cdk2	LYS	237	NZ	3.55	Cks1	GLU	61	OE1
Cdk2	LYS	242	N	3.09	Cks1	ASP	14	OD2
Cdk2	TRP	243	NE1	2.78	Cks1	ASP	13	O

Table 3 details the intermolecular hydrogen bond interactions in complex Cdk2-Cks1 between two amino acids (donor and acceptor) of differing proteins at the complex interface, a total of 7 hydrogen bonds.

donors and acceptors as well as the distance between heavy atoms. Complex Cdk2-Cks1 includes the highest amount of hydrogen bonds of the three complexes. The majority being backbone hydrogen bonds. The same observation is found in the Cdk1-Cks1 complex in smaller quantities. The Skp2- p27^{Kip1}-Cks1 complex has the most variation between side chain and backbone hydrogen bonds.

Computational Alanine Scans

The $\Delta\Delta G$ values (kJ/mol) for each ionizable amino acid when mutated to alanine, out of the range of thermal fluctuations (± 2.5 kJ/mol), is listed in **Table 4** respective to each protein, one letter amino acid code, sequence number and mutation to alanine. A negative $\Delta\Delta G$ value indicates a gain-of-binding mutation while a positive $\Delta\Delta G$

value corresponds to a loss-of-binding mutation relative to the parent sequence. There were 13 total residues that when mutated were out of the standard range of thermal fluctuations in the Cks1-Skp2- p27^{Kip1} complex, split evenly between Skp2 and Cks1 with one mutation on p27^{Kip1}. All of the $\Delta\Delta G$ values for residues on Skp2 were positive while Cks1 had equal amounts positive and negative and the one value for p27^{Kip1} was positive. A majority, 11 of the 14 mutations recorded in the Cdk1-Cks1 complex were on the Cdk1 chain and had negative $\Delta\Delta G$ values. Three residues from Cks1 were recorded, one with a high negative $\Delta\Delta G$ value and the other 2 were positive. The Cdk2-Cks1 complex had two residues, LYS 237 and ARG 217 that displayed $\Delta\Delta G$ values out of the normal range and were both positive.

Cks1-Skp2- p27 ^{Kip1}			Cdk1-Cks1			Cdk2-Cks1		
Protein	Mutation	$\Delta\Delta G$	Protein	Mutation	$\Delta\Delta G$	Protein	Mutation	$\Delta\Delta G$
Skp2	R164A	2.80	Cdk1	E232A	-7.02	Cdk1	K237A	3.65
Skp2	R344A	3.81	Cdk1	E209A	-5.67	Cdk1	R217A	3.81
Skp2	R294A	5.18	Cdk1	E163A	-5.23			
Skp2	R167A	5.40	Cdk1	D207A	-4.89			
Skp2	R398A	5.50	Cdk1	E173A	-4.80			
Skp2	D347A	5.97	Cdk1	D236A	-4.69			
Cks1	K34A	-3.15	Cdk1	D211A	-3.71			
Cks1	E40A	-3.02	Cdk1	K266A	2.62			
Cks1	E16A	-2.62	Cdk1	R218A	3.90			
Cks1	D10A	3.27	Cdk1	K243A	4.61			
Cks1	R70A	4.69	Cdk1	K238A	5.47			
Cks1	R44A	4.94	Cks1	E61A	-11.18			
p27 ^{Kip1}	E185A	4.78	Cks1	D14A	2.65			
			Cks1	R70A	4.89			

Table 4 displays the results from the AESOP alanine scan for each complex respective to the protein, initial one letter amino acid code, sequence number, mutation to alanine and the resulting $\Delta\Delta G$ (kJ/mol).

Electrostatic Surface Potentials

The electrostatic surface potentials produced by APBS are juxtaposed to the corresponding contact region, both displayed **Figures 1-3** in the open book conformation. The Skp2-p27^{Kip1}-Cks1 complex displays marginal, dispersed electrostatic complementarity between Skp2 and Cks1 at the contact region. In the Cdk1-Cks1 complex, there is a negative charge contact region on Cks1 that corresponds to a positively charged area on Cdk1. The Cdk2-Cks1 complex includes a negative charged region on Cks1 that appears to bind directly to a positive charge site on Cdk2.

Molecular Dynamics

The heat maps for the occupancy, or percentage of occurrence over the course of the simulation trajectory, of the interactions between Cks1 and p27^{Kip1} s are shown in **Figure 4** indicated by a color scale from 0.0 (0%) to 1.0 (100%) from blue to red, respectively. Multiple key residues with high rates of interaction occurrence throughout the molecular dynamics trajectory are: ARG 20, ARG 44, GLN 49, GLN 50 and SER 51 on protein Cks1. The corresponding residues and atoms on p27^{Kip1} involved in those interactions can be a target for further

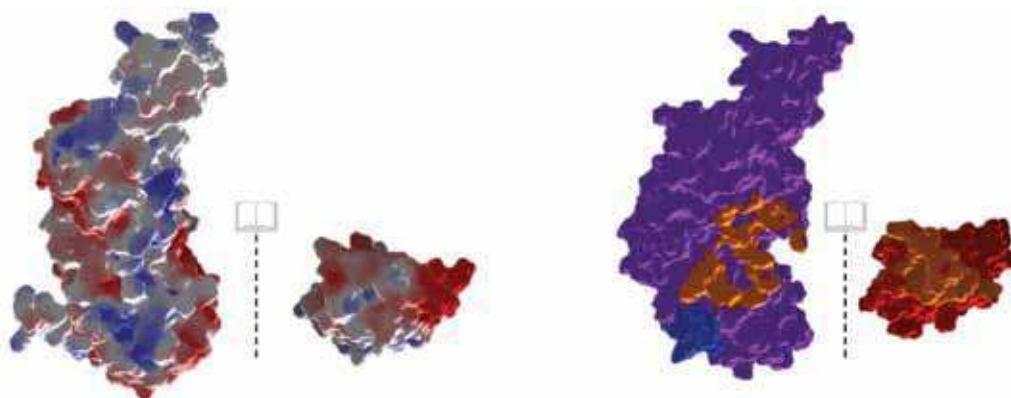


Figure 1 displays the electrostatic projection of proteins Skp2-p27^{Kip1}-Cks1 by the range of $-6kT/e$ to $+6kT/e$ colored red to blue respectively with white indicating no charge. Compared to the colored surfaces distinguishing separate chains, with contacts highlighted in orange and protein Skp2 colored purple, p27^{Kip1} colored blue and Cks1 colored red.

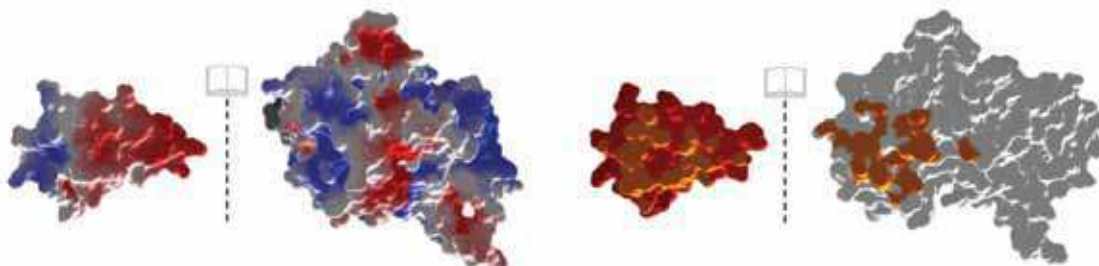


Figure 2 displays the electrostatic projection of proteins Cdk1-Cks1 by the range of $-6kT/e$ to $+6kT/e$ colored red to blue respectively with white indicating no charge. Compared to the colored surfaces distinguishing separate chains, with contacts highlighted in orange and protein Cdk1 colored white and Cks1 colored red.

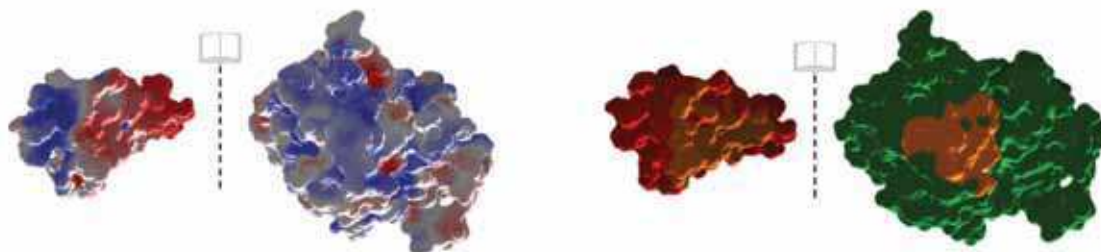


Figure 3 displays the electrostatic projection of proteins Cdk2-Cks1 by the range of $-6kT/e$ to $+6kT/e$ colored red to blue respectively with white indicating no charge. Compared to the colored surfaces distinguishing separate chains, with contacts highlighted in orange and protein Cdk2 colored green and Cks1 colored red.

investigation of creation of a disruptive biotherapeutic. Additionally, the heat map for the occupancy of hydrogen

bonds confirms the importance of residues: ARG 20, ARG 44 and SER51 in complex stability.

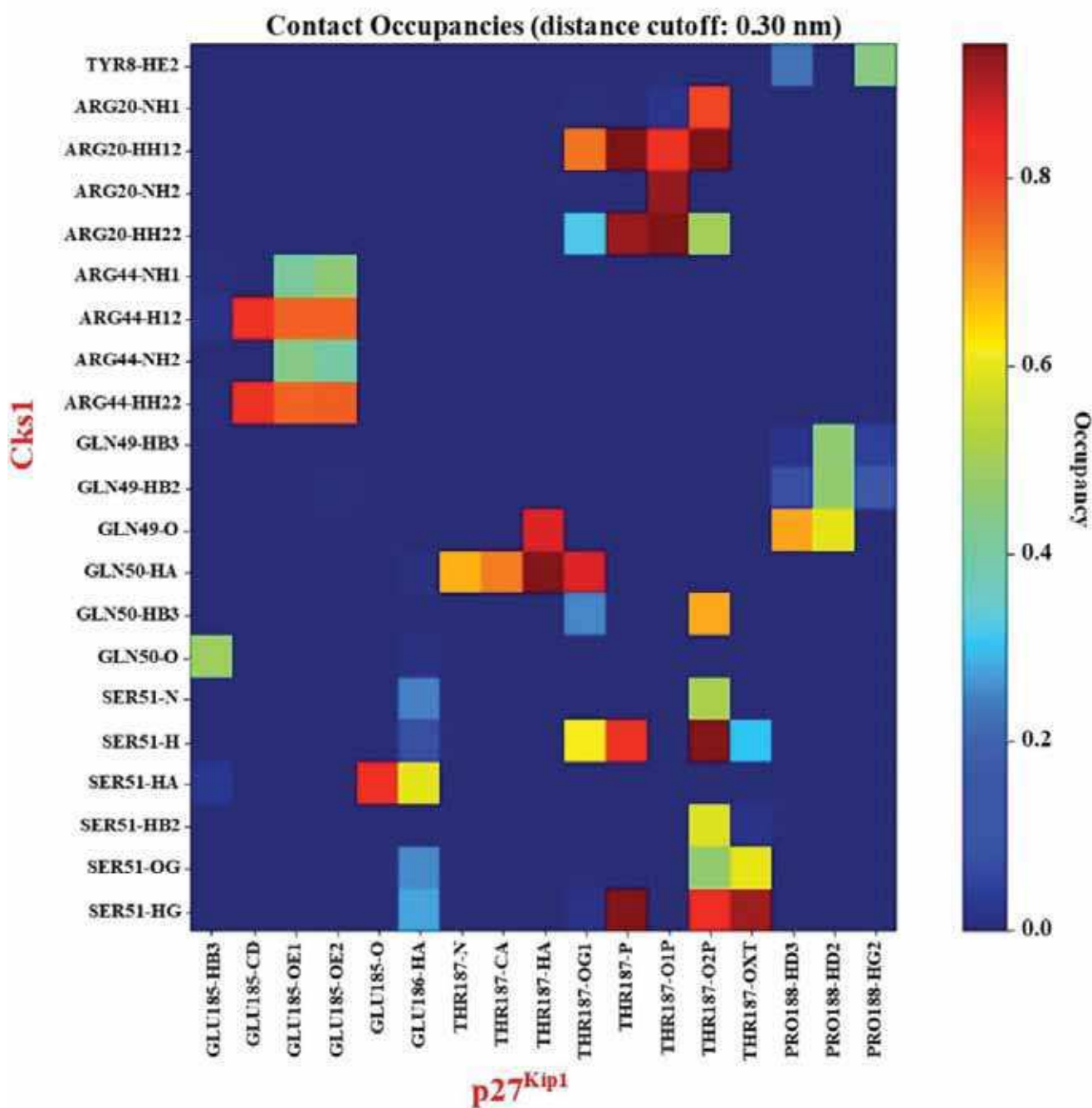


Figure 4 displays the occupancy of interactions throughout the trajectory of the molecular dynamics simulation. Interaction sites are labeled according to the three letter amino acids code, sequence number and atom identifier with protein Cks1 on the left, vertically aligned and protein p27^{Kip1} labeled on the bottom, horizontally

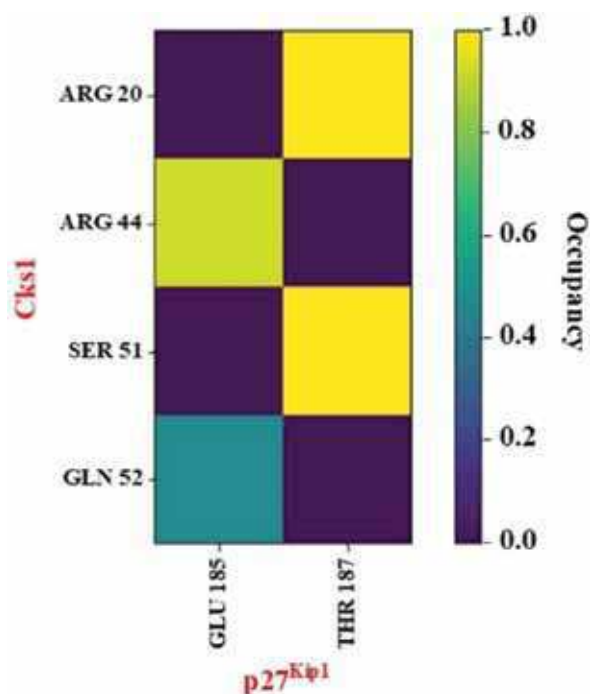


Figure 5 displays the occupancy of hydrogen bonds throughout the trajectory of the molecular dynamics simulation. Interaction sites are labeled according to the three letter amino acids code, sequence number and atom identifier with protein Cks1 on the left, vertically aligned and protein p27^{Kip1} labeled on the bottom, horizontally.

DISCUSSION

Intermolecular Hydrogen Bonds

Based on the hydrogen bond tables for each respective complex, all of the bonds are within the typical 3.5 Å range suggesting that these interactions are strong and contribute to the stability of the complex. There is variety between the complexes with distribution of backbone and side chain hydrogen bonds. The Cks1-Cdk1 complex having all backbone hydrogen bonds and Cks1-Cdk2 having the highest amount of total bonds in addition to the majority being to the backbone. The Skp2- p27^{Kip1}-Cks1 complex had the most diversity of types of hydrogen bonds between side chains and the backbone. Overall, with the hydrogen bonds of each system characterized by the optimal distance, angle and distribution of backbone and side chain bonds the stability of each interaction region may be difficult to disrupt.

Electrostatic Surface Potential

In Skp2-p27^{Kip1}-Cks1, translating the region of contact to the APBS electrostatic surface map shows there is a

small amount of scattered electrostatic complementarity. However, there appears to be no condensed, direct complementarity at the interface suggesting that charged interactions may not play a strong role in complex stability. In Cks1-Cdk1, there potentially is direct charge complementarity at the binding interface. A small pocket of negative charge on Cks1 appears to correspond to a region of positive charge at the contact region on Cdk1 leading to suggest that these charged interactions assist in structural stability. Finally, in Cks1-Cdk2 there is no apparent direct electrostatic complementarity. The contact region on Cks1 has a negative charge and Cdk2 has an accompanying weak, positive region at the contact zone offering some reliance of the system of electrostatics for stability.

Computational Alanine Scans

Observing a direct complementarity of mutations across the complex lends to a strong interaction that can add to the stability of the complex. In Skp2- p27^{Kip1}-Cks1 there are a majority of positive, loss-of-binding mutations which happen to be in close physical proximity to each other, verified manually in Chimera. However, while there are several gain-of-binding mutations none occur in the same region as the loss-of-binding mutations. Then for Cks1-Cdk1 there is a majority of gain-of-binding mutations which are close in physical proximity. However, again there is not direct complementarity shown through the mutations and when verified in Chimera. Finally, in Cks1-Cdk2 only two ionizable amino acids are outside the effects of thermal fluctuations indicating that electrostatics may not play an important role in the complex stability.

Molecular Dynamics

The heat maps composed of the occupancies of interactions produced by molecular dynamics offers a vast amount of information regarding potential areas of targeted therapeutics. Refining down to the key interactions with high occupancies, a potential targeted attribute profile can be created to mimic these characteristics. In particular, pTHR (phosphorylated threonine) 187 on p27^{Kip1} is involved in multiple high occupancy interactions. Thus, to inhibit interactions between p27^{Kip1} and Cks1, one could attempt to prevent such interactions. Additionally, GLU 185 on p27^{Kip1} offers another source for interruption due to the large amount of hydrogen bonds between Arginine 44 on Cks1. Varying emphasis between these residues

and others at the binding pocket can lead to potential therapeutics that bind competitively to p27^{Kip1} disrupting the dysfunctional cycle in cancer cells.

CONCLUSION AND PERSPECTIVE

Through the quantitative results of the hydrogen bond analysis, there are significant regions of side chain and backbone bonds stabilizing Cks1 to adjacent proteins in each of the three complexes. Additionally, through the qualitative assessment of comparing electrostatic surface potentials to the contact region and quantitative results from AESOP, charged interactions at the interface were most prevalent between the Cks1-Skp2 and Cks1-Cdk1 complexes. Through the computational evaluation of electrostatic relevance and hydrogen bonding to the stability of the three complexes, the broad contact regions between Cks1 and Skp2, CDK1, CDK2 had potential,

but were not prime targets for disruption. The large binding interfaces utilized strong hydrogen bonding and electrostatic complementarity to stabilize the complex which would potentially be difficult to disrupt. However, due to the relatively small and condensed binding region between phosphorylated p27^{Kip1} and Cks1, additional categorization and ranking of all possible methods of interaction is an area of future research opportunity. This led to the fine-grain analysis performed by molecular dynamics of the Cks1-p27^{Kip1} system. The study revealed key interactions that occur with high frequency, notably the hydrogen bonds and charged interactions involving pTHR 187 and GLU 185 on p27^{Kip1} which target SER 51, ARG 20, GLN 49 and GLN 50 on Cks1. These interactions are prime targets for developing new inhibitors for interactions between p27^{Kip1} and Cks1 which can lead to new cancer therapies that restore cell cycle regulation.

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