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UNIVERSITY OF CALIFORNIA, RIVERSIDE

UNDERGRADUATE RESEARCH JOURNAL

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TABLE OF CONTENTS

UNIVERSITY OF CALIFORNIA, RIVERSIDE

UNDERGRADUATE RESEARCH JOURNAL

TABLE OF CONTENTS (CONT'D)

FROM THE CHANCELLOR

As a research university, one of UC Riverside's most important duties is the creation of knowledge. Creating an environment and structure that fosters innovation to solve our community and world's greatest challenges is in our university DNA.

With faculty-mentored research projects across a breadth of disciplines, UCR provides a wealth of opportunities for students to investigate complex questions while building research skills. Each year, I am excited to see how students have embraced this part of their education while further developing their own scholarly interests.

In this 18h volume of the UC Riverside Undergraduate Research Journal, our students are making the most of these opportunities and accomplishing truly inspiring work. From knee health to procrastination, haptic search to food insecurity, this edition of the Journal covers significant scientific ground while showing research excellence and creative endeavors of the highest order. Yet, the Journal has something more to offer than discovery. It seems that hope for a future led by these bold and brilliant minds is the true gift within these pages.

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

Sincerely,

Kim A. Wilcox
Chancellor

FROM THE VICE PROVOST AND DEAN

Whether a student is interested in the humanities, arts, social sciences, policy issues, education, business, agricultural, or the natural sciences, students bring a commitment to rigor, relevance, and knowledge-creation to the creative and research endeavor—commitments that are captured by the articles within this journal. Such work is only possible when students are surrounded by a community of peers and faculty mentors who are dedicated to the pursuit of knowledge—institutional characteristics that are no doubt present and thriving at UC Riverside.

Undergraduate student research is the beginning of a journey driven by student curiosity about a topic and their commitment to exploring it further. When embarking on a new topic, researchers, and emerging researchers, are often inspired to ask and develop well-formulated questions; explore the work that has already been done in the topic area; identify a set of methods that coincides with the research question(s); and work diligently to create, generate, or collect data to help answer the question(s). In the university context, students are often driven toward a topic that sparks their interests, passions, and commitments to a discipline, community, or problem they are trying to address in the world.

The student-authors in this issue of the Journal demonstrate the benefits of living, learning, and engaging in research at a research-intensive university. In fact, to be surrounded by scholars who engage in cutting-edge research and scholarship is one of the greatest benefits of being at a university like UC Riverside. The student-authors in this journal have taken full advantage of the opportunities in front of them and should be applauded for sharing their work with the world of research, which extends far beyond UC Riverside.

I congratulate all of the student-authors and faculty mentors who made this research possible, and I look forward to seeing their future contributions.

Sincerely,

Louie F. Rodriguez
Vice Provost and Dean, Division of Undergraduate Education
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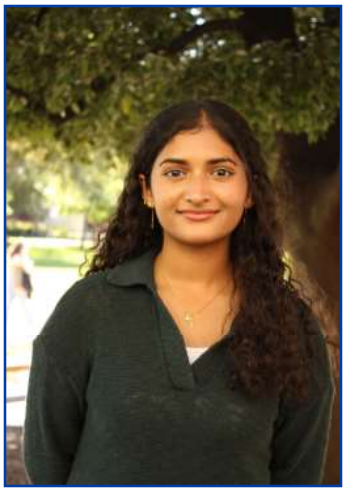

Angelin Simon
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It is with great pleasure that we present UC Riverside's 18th edition of the *Undergraduate Research Journal*. The *Journa*l encapsulates the 4 pillars of UCR's Tartan Soul: Excellence, Respect, Integrity, and Accountability. We are excited to publish the cumulative efforts of our students' brilliance, persistence, and patience. This issue of the *Journal* represents the continuous hard work and dedication of the Student Editorial Board and the Faculty Advisory Board. We thank the authors for their commitment to ensuring the endless possibilities of the future in research. Your achievements, found in this edition, will forever be a part of the *Journal*'s legacy. We also thank our Student Editorial Board and Faculty Advisory Board—your diligence and dedication to the publication process have ensured the quality and success of the 18th edition of the *Journal*. We are grateful to have been part of the outstanding team that made this edition possible.

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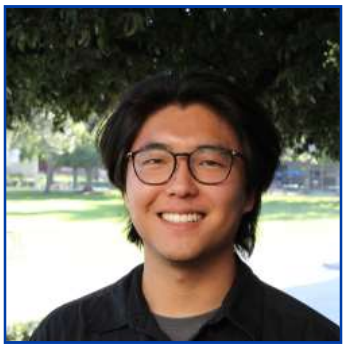

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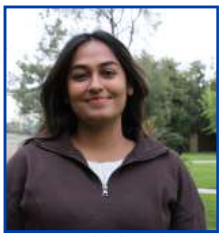

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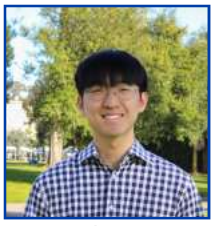

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Since UCR's Undergraduate Research Journal started 18 years ago, it has published over 175 scholarly articles across many fields. These papers represent the commitment of our undergraduate students to performing independent research as part of their undergraduate experience. Because undergraduate research can often form part of a larger work with many contributors, the importance of undergraduate students' contribution can sometimes be lost. With the Undergraduate Research Journal, students can publish their work as first authors before the end of the academic year through a peer-review publication process. The paper becomes a part of students' professional experience, contributing to their record of scholarly achievement. The Journal's submission and review process is run by undergraduates who form the Student Editorial Board, working with members of the Faculty Advisory Board. We owe a debt of gratitude to these students for their professionalism and dedication to the review and preparation of the articles in this issue. We are also grateful for the participation of the members of the Faculty Advisory Board in guiding the reviewers. I would like to thank Jennifer Kavetsky for her work in supporting and guiding the process, Gladis Herrera-Berkowitz and the CUREL team, as well as Undergraduate Education for their funding support. If you are interested in publishing your undergraduate research at UCR, consider submitting to our next issue!

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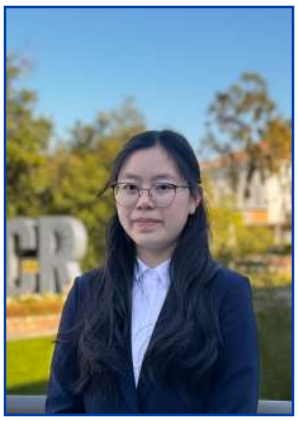

MANDY HSIEH

Mandy Hsieh is a third-year Bioengineering major with a minor in Data Science. She is involved in research as a member of the Biotransport and Bioreaction Kinetics (B2K) Group under Dr. Victor G. J. Rodgers. Additionally, she contributes to the chemical synthesis and bioprinting team led by Dr. Iman Noshadi in the Innovative Biomaterials Laboratory, where she received a mini-grant award. Mandy also leads the Power Team in AIChE's ChemE Car Competition and is passionate about pharmaceuticals, regenerative medicine, tissue engineering, and biophysics. In her free time, she enjoys sightseeing, drawing, and photography.

This is a Japanese Sea Nettle, taken at the Aquarium of the Pacific. Despite their delicate appearance, jellyfish symbolize fluidity, adaptability, grace, and resilience through their capability of navigation in uncertain situations. Their tranquil movements serve as a reminder to embrace change, to have faith in our abilities, and to be resilient in the ever-changing tides of life.

Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

Lorenzo Bazzani, Department of Molecular, Cell, and Systems Biology
Erica Heinrich, Ph.D., Department of Biomedical Sciences

ABSTRACT

The knee joint faces daily stresses that cause its overall health to degrade and pathologies to develop. I hypothesized that increased stress on the knee joint and imbalance in thigh musculature would positively correlate with increased acoustic emissions from the knee joint, a biomarker of inflammation in the joint. We tested this hypothesis by selecting a cohort of healthy, moderately active individuals aged 18-32 across a range of BMIs. We collected baseline knee acoustic measurements and measured quadricep and hamstring flexibility, hamstring and quadricep maximum voluntary isometric contraction, and heel strike angle during self-selected walking gait. Heel strike angle does not correlate with increased acoustic emissions from the knee, but BMI negatively correlates with the hamstrings:quadriceps strength ratio. Furthermore, left hamstring flexibility positively correlates with left heel strike angle. Finally, we found that right quadricep flexibility had a positive correlation with right heel strike angle. Since the hamstrings:quadriceps strength ratio is an important biomarker for knee health, this finding may indicate evidence of progressing knee pathology in individuals with higher BMI. Changes in gait associated with muscle rigidity indicate that differential levels of upper leg muscle flexibility may translate to changes in the mechanics of everyday movements, such as walking.

KEYWORDS: knee health, BMI, strength, stability

FACULTY MENTOR - Dr. Erica Heinrich, Department of Biomedical Sciences

Dr. Erica Heinrich is an Assistant Professor at the UC Riverside School of Medicine, Division of Biomedical Sciences. She trained as a comparative physiologist and currently studies the control of breathing and inflammatory signaling in human populations at high altitude.

LORENZO BAZZANI

Lorenzo Bazzani completed his Bachelor of Science in Cell, Molecular, and Developmental Biology, Magna Cum Laude, in June 2023. In 2022, he began researching physiological systems in the Heinrich Lab. Using the knowledge and questions sourced from his time as a hospital volunteer and scribe, he designed and conducted his Honors Capstone under Dr. Erica Heinrich's supervision. He is now a medical assistant at a sports medicine clinic while applying to medical schools.

Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

INTRODUCTION

The knee joint is a complex weight-bearing joint used extensively in everyday life. Its stability is maintained by ligaments, cartilage, and the muscles surrounding the joint. Due to its frequent use, the joint is prone to a myriad of painful conditions. Among the most common of these conditions is osteoarthritis (OA). OA most often affects weight-bearing joints and is characterized by pain, limitations in range of motion, and muscular weakness (Coaccioli et al., 2022). As of now, the only cure for OA is reconstructive surgery. Other therapies focus on managing the symptoms associated with the condition. OA has long been regarded as a relatively simple condition that arises due to chronic overuse. However, recent findings indicate that factors related to metabolic and cardiovascular health contribute to osteoarthritis development. These include chronic joint inflammation, cholesterol imbalances in the blood, and blood vessel dysfunction (Coaccioli et al., 2022). Therefore, understanding the progression of knee health throughout an individual's life may provide enhanced preventative treatment options for knee pathologies. The hope is that if factors that exacerbate knee OA are present in a younger individual, these may be corrected before the disease has progressed significantly.

A critical factor in the knee joint's function is the hyaline cartilage, which coats the articular surfaces of synovial joints (Nahian, 2022). It allows for the femur and tibia to glide across each other smoothly, facilitating movement and absorbing impact. Cartilage is a tissue with little nutrient supply from blood vessels and is composed of chondrocytes and the cartilaginous matrix. Unlike other cells, chondrocytes respond to increased stress by dying, which reduces cushioning and increases stress on the underlying bone. Bone cells, conversely, respond to increased stress by producing more bone (Nahian, 2022). This bone modification, coupled with chondrocyte death, transforms the once-smooth articular surface into a rough and poorly cushioned environment. These rough surfaces grinding together cause significant pain in individuals with OA.

The knee joint may face increased stress during locomotion due to abnormal gait patterns. Studies indicate that increased heel strike angle increases the force exerted on the knee joint during walking (Levinger et al., 2008). Repetitive, increased stress contributes to cartilaginous degradation and subchondral bone modification (Hurley, 1999). Therefore, gait analysis may provide valuable insight into knee OA progression.

Thigh musculature also contributes to knee stability, especially during movement. The thigh musculature includes quadricep muscles in the front of the thigh, which contract to straighten the knee, and hamstring muscles in the back of the thigh, which assist in bending the knee. Evidence suggests that weaker thigh muscles stabilize the knee joint less effectively, therefore increasing stress on cartilage during locomotion and movement (Hurley, 1999). Multiple studies indicate that while quadricep weakness is frequently present in those with knee OA, it is not always secondary to pain. Weakness may therefore be both a predictive and etiologic factor (Øiestad et al., 2015). Hence, it is reasonable to suspect that thigh muscle characteristics contribute to knee joint health.

Knee acoustic emissions (AEs) have been utilized as convenient, cost-effective, and non-invasive biomarkers to objectively quantify the state of the knee joint (Yiallourides et al., 2021). Unlike X-rays or magnetic resonance imaging (MRI), AEs do not require large, costly machines. Furthermore, AEs are quickly and easily measured using small microphones placed on the surface of the skin. Studies indicate that increased peak and average knee AEs during flexion and extension suggest progressed pathology (Yiallourides et al., 2021). Hence, this study will include the use of knee AEs to examine knee health.

The goals of this research project are (1) to determine if hamstrings:quadriceps (HQ) strength ratio correlates with biomarkers of knee health, including knee AEs, and (2) to determine if gait patterns, measured by heel strike angle, correlate with increased knee AEs. I hypothesize that a larger HQ ratio and a larger heel strike angle will both demonstrate a positive correlation with increased AEs from the knee.

Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

METHODS

Ethical statement:

Experiments were approved by the UC Riverside Clinical IRB (HS 22-064). All work was conducted according to the *Declaration of Helsinki,* except registration in a database. Participants were provided informed consent, including the benefits and risks of study participation, in their native language (English) before participation.

Study participants:

27 moderately active, healthy university students (16 males, 11 females) with a mean age of 22 years (SD: 3.85 years) were recruited in the winter of 2023 (Table 1). Exclusion criteria included: age greater than 35; history of major leg injury, surgery, or pain; elite athletes; history of neuromuscular impairment or disease; current knee or leg pain; and lack of English fluency. Prior to their appointment, participants were asked to shave the areas of microphone placement and wear shorts and form-fitting clothing.

Variable	Men (N=16)	Women (N=11)
Age (years)	22.19 (4.02)	21.91 (3.58)
Height (cm)	177.05 (6.93)	161.35 (4.35)
Weight (kg)	78.66 (12.77)	59.52 (6.42)
Body Mass Index (kg/m^2)	25.08 (3.46)	22.96 (3.18)

Table 1. Participant demographics. Data are represented as means (standard deviations).

Study design:

Participants completed a knee evaluation questionnaire (2000 IKDC Subjective Knee Evaluation Form) and a lifestyle/demographics questionnaire prior to participation (Anderson et al., 2006). We then measured physiological parameters including height, weight, and blood pressure. Following these procedures, participants completed a series of maneuvers in the following order: knee audio emission, flexibility measures, strength measures, and gait analysis.

Knee acoustic emission:

Two cardiac microphones were placed on the medial and lateral aspect of the right knee joint (MLT201, ADInstruments, Dunedin, FL, USA) and secured using cloth medical tape and a knee brace, as depicted in Figure 1. Hair removal was performed with a razor at the site of microphone placement if necessary to ensure accurate acoustic emission recording. The microphone detected audio signals and sent these to a data interface (Powerlab 8/35, ADInstruments) which transcribed digital voltage measures to analog data which was collected using LabChart 8 software (ADInstruments). Subjects were instructed to perform five seated knee extensions, resting for at least one second between each repetition. After this, subjects were instructed to perform five sit-to-stand repetitions, again resting for at least one second between each repetition. This procedure was repeated for the left leg. To quantify AEs from the knee joint, peak and mean signal amplitudes were measured during each movement in LabChart 8.

Figure 1. Experimental setup for the knee acoustic emission portion.

Flexibility measures:

Angles were measured using a 12" goniometer (Ever Ready First Aid, Brooklyn, NY). Quadricep flexibility was measured using a modified Thomas test (Harvey, 1998) Briefly, each participant was instructed to lie on their back and bring one knee to their chest, grasping their shin with both hands. Each was instructed to relax the opposite leg while keeping their lumbar spine in contact with the bench during the entire maneuver. Once the participant confirmed their leg was relaxed, the popliteal joint angle was measured. Then, the testing knee was flexed until they reported discomfort, and the joint angle was re-measured.

We measured hamstring flexibility with a passive knee extension test (Gnat et al., 2010). The participant was placed on the bench with their hip flexed at 90 degrees. They were

then instructed to extend their knee as far as they voluntarily could, and the popliteal joint angle was measured. We then further straightened the participant's knee until they reported discomfort and re-measured the joint angle.

Strength measures:

Maximal voluntary isometric contraction (MVIC) measures were determined for both the quadriceps and the hamstrings. Surface electromyography (EMG) probes were placed on participants' rectus femoris muscles. The participants were seated upright on an exercise bench with their knees at a 90-degree angle. The exercise bench was anchored in place to prevent movement. The participants were then instructed to maximally contract their quadriceps for three seconds, timed using a stopwatch. Their knees were reset to 90 degrees between trials and the left and right legs were tested separately. The hamstring MVIC test followed quadricep testing. EMG probes were placed on the semitendinosus muscle. The participants were placed supine with one knee on the bench at 90 degrees and the opposite foot resting on the ground for support. They were then instructed to maximally contract their hamstrings for three seconds, timed using a stopwatch, after which they were allowed to relax their hamstrings to minimize unwanted fatigue. During each contraction, peak force production was measured using a scale that measured maximum force output (Klau OCS-L Weighing Scale). MVIC measures were conducted in triplicate and averaged for each leg.

Gait analysis:

The participants removed their shoes, and we placed high-contrast markers on various aspects of the subjects' feet, legs, and hips. Markers were placed on the greater trochanter of the femur, the lateral knee joint line, the lateral malleolus of the ankle, the lateral calcaneus, the distal aspect of the fifth metatarsal, the first distal phalange, the distal aspect of the first metatarsal, and on the tibialis anterior tendon. The participants were instructed to walk at their preferred walking speed in a straight line on a solid ground surface. During this maneuver, high-speed video recordings of the hip joints and below were recorded (SC1 High Speed Video Camera, Edgertronic, San Jose, CA, USA). The participants were instructed to fixate on a point at eye-level straight in front of them and rest their arms on their shoulders. After they walked through once, they were instructed to repeat the same procedure, but facing the opposite way.
To calculate heel strike angles, ImageJ (LOCI, University of Washington, WA) was used. The video frame in which the heel first contacted the floor was chosen for analysis. Heel strike angle was calculated by using the angle function and measuring from a perfect level, determined by the y-value in the image used as well as points R4/L4 and R5/L5.

Statistical analyses:

All statistical analyses were performed in R Studio (R version 4.2.2). To determine the relationship between knee joint AEs and our variables of interest (heel strike angle and HQ ratio), we first checked the normality of each variable's distribution using a Shapiro-Wilks test for normality and visualization via Q-Q plots. If the assumption of normality was met, Pearson correlations were performed using the stat_cor function in the *ggpubr* package in R to examine linear relationships between these variables. We also conducted a large-scale correlation analysis of all variables of interest using the *rcorr* function in the *Hmisc* package in R. To adjust for the impact of potential covariates on our outcome of interest, we performed general linear model analyses with age, sex, and BMI as covariates using the *lm* function in R.

RESULTS

Healthy university students (N=16 men, 11 women) between 18 and 32 years of age (22 ±3.8 years) were recruited for this study. Figure 2 shows a negative correlation between HQ strength ratio and BMI on the right leg with a similar trend on the left leg. N=20 participants reported that they were right-leg dominant.

To determine if BMI and HQ ratio were related, Pearson correlation coefficients were calculated for both the left and right HQ ratios. Correlation coefficients in Figure 2 indicate that BMI has a negative relationship with HQ ratio.

To determine if HQ strength ratio and knee acoustic emission were related, we performed a Pearson correlation analysis comparing heel strike angle with the mean and peak amplitude of AEs from the microphones placed on both

Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

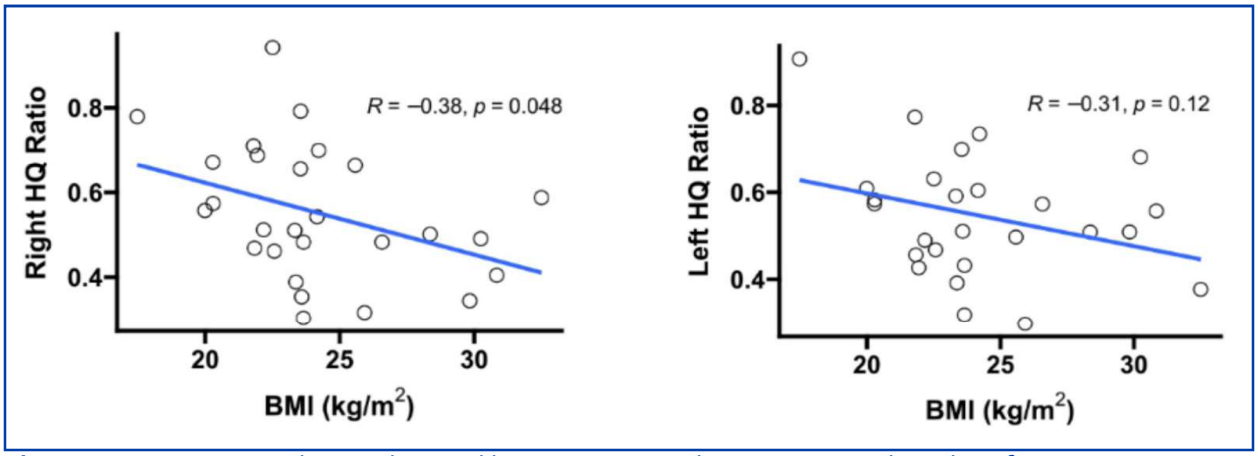

Figure 2. Negative correlation observed between BMI and HQ ratio. R and p values for a Pearson correlation analysis are provided. Each point represents data from a single participant, with one measurement plotted per individual.

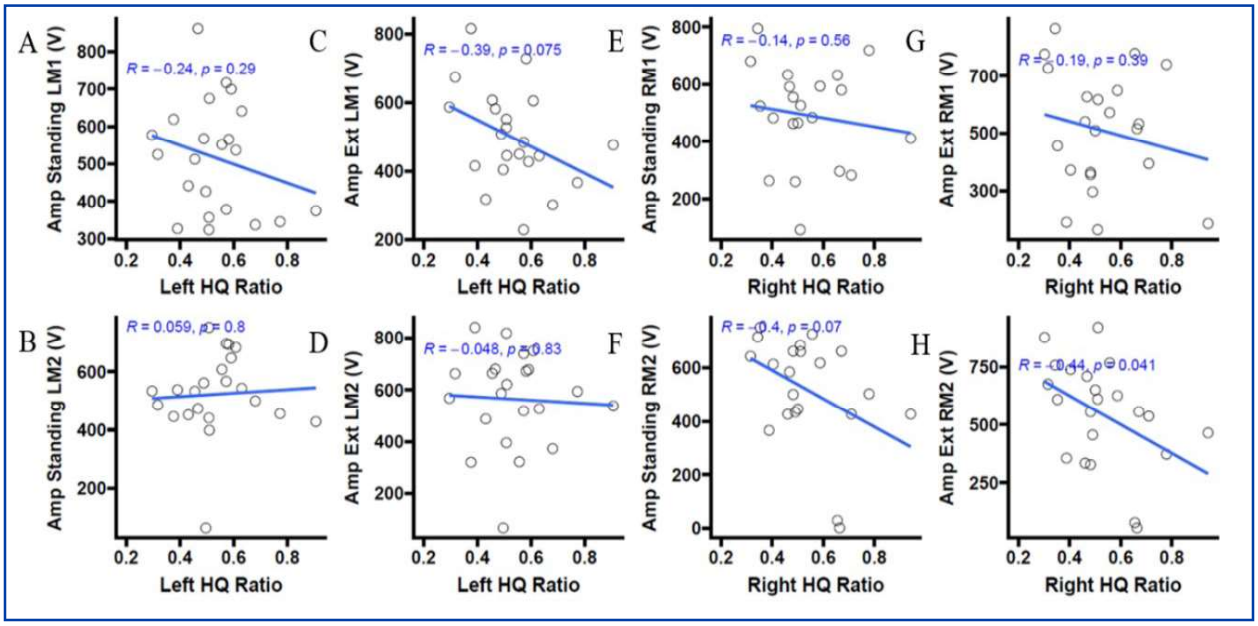

Figure 3. Results for average acoustic emission from the knee joint and HQ strength ratio. Plots represent data collected during seated knee extension (B, D, F, H) or standing from a seated position (A, C, E, G). Data is recorded from a microphone located on the medial joint line (M1) and the lateral joint line (M2). R and p values from a Pearson correlation analysis are provided in blue text. Each data point represents a measure from a single participant, with one measurement plotted per individual.

Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

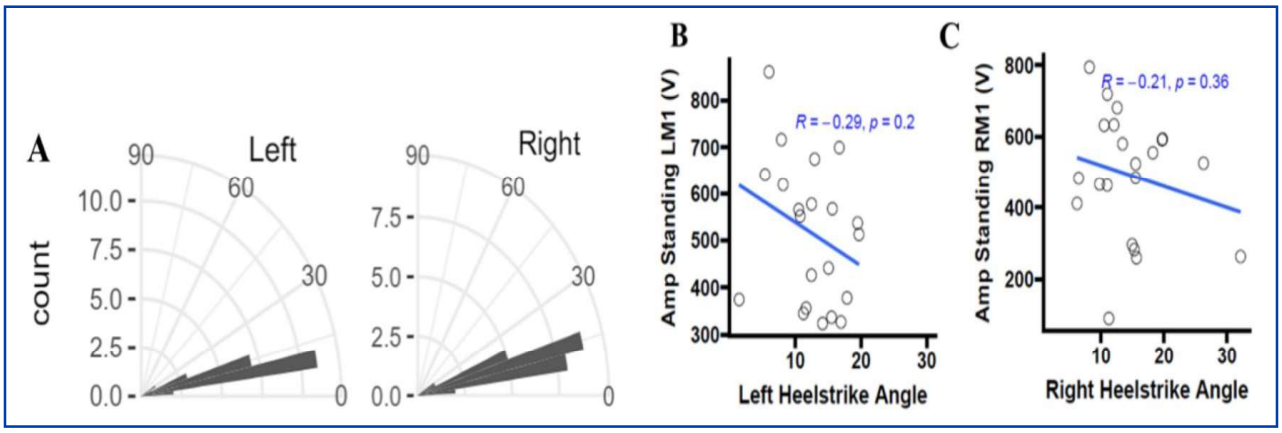

Figure 4. Representative results for average acoustic emission from the knee joint and heel strike angles. Histogram showing the distribution of heel strike angles across participants (A). Representative data from one microphone position compared to heel strike angles from left foot (B) and right foot (C). R and p values from a Pearson correlation analysis are provided in blue text. Each data point represents a measure from a single participant, with one measurement plotted per individual.

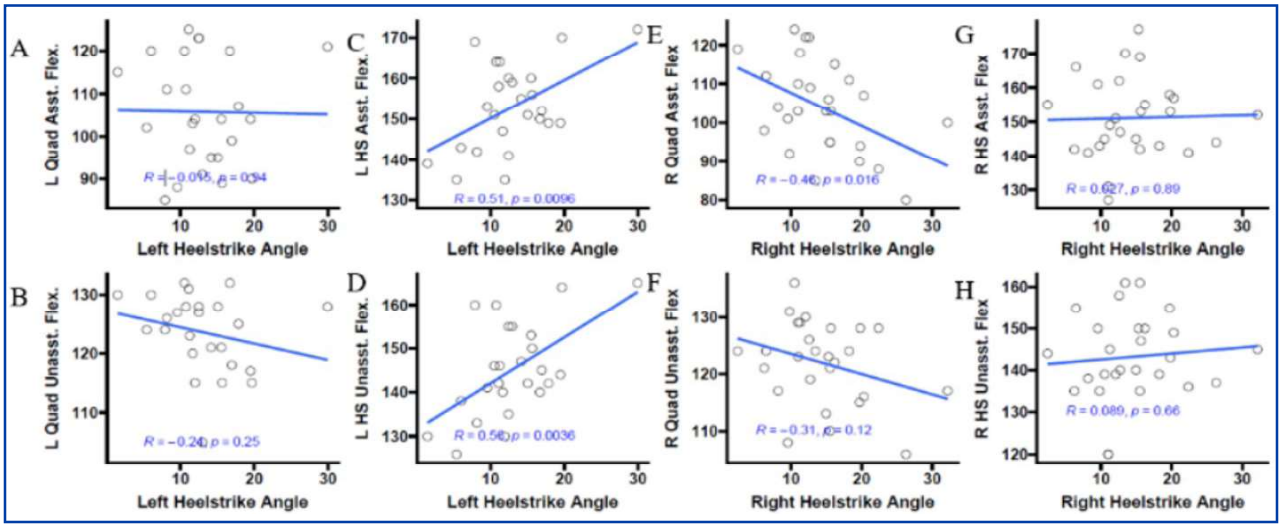

Figure 5. Representative results for quadricep/hamstring flexibility and heel strike angles. Plots represent data collected during either the modified Thomas test (A, B, E, F) or during the passive knee extension test (C, D, G, H). For hamstring flexibility measures, larger angles represent increased flexibility. For HQ flexibility measures, larger angles represent decreased flexibility. R and p values from a Pearson correlation analysis are provided in blue text. Each data point represents a measure from a single participant, with one measurement plotted per individual.

the medial and lateral joint line. There may be a relationship between decreased HQ strength ratio and reduced AEs from the knee (Figure 3H). To determine if heel strike angle and knee AEs were related, we performed a Pearson correlation analysis between the two variables. Heel strike angle may have a weak relationship with mean AE amplitude from the knee (Figure 4).

We performed Pearson correlation analyses to determine if quadricep and hamstring flexibility had any relationship with heel strike angle. We compared both hamstring flexibility and quadricep flexibility (assisted and unassisted) to heel strike angle. Left hamstring flexibility (both assisted and unassisted) has a strong positive correlation with left heel strike angle (Figure 5C, D). Right assisted quadricep flexibility has a strong positive correlation with right heel strike angle (Figure 5E). Unassisted right quadriceps flexibility showed a weaker correlation with right heel strike angle (Figure 5F).

DISCUSSION

The goal of this study was (1) to determine if the HQ strength ratio relates to biomarkers of knee health, including knee AEs, and (2) to determine if gait patterns, quantified by heel strike angle, are associated with increased knee AEs. Our findings corroborate much of the existing literature regarding the relationship between obesity and an increased risk of eventual OA (Pottie et al., 2006). However, such a correlation between HQ strength ratios and BMI was unexpected in the cohort studied here, considering that the study employed healthy, moderately active young adults. It has long been established that individuals with higher BMIs face increased stress on the knee joint. However, this study suggests that the negative effects of this stress are exacerbated by imbalances in the HQ ratio. Hence, not only do individuals with a higher BMI place increased stress on their knees, their imbalanced musculature also renders the joint less stable. This instability can exacerbate the progression of knee OA if left uncorrected.

The mechanism behind the negative correlation between BMI and HQ ratio remains unclear. The quadricep muscles are more involved than the hamstring muscles in walking (Hurley, 1999). Since walking is the most common form of human locomotion, we hypothesize that individuals with higher BMI may perform comparatively more of this exercise versus exercises that target the hamstrings more directly. This observed pattern may also be a question of differential rates of atrophy between the two muscle groups. Currently, there is little data that examines how BMI and HQ ratio are associated in healthy young adults. As such, future work will investigate the observed trend. Future work may also incorporate more accurate measures of body fat percentages, such as DXA scans or skinfold calipers, to better elucidate the relationship between body mass and HQ ratio.

While we expected to see a relationship between heel strike angle and knee joint AEs, there was no significant correlation between these two variables. Hence, we cannot conclude that increased heel strike angle is associated with declines in knee health in this cohort. However, heel strike angle is just one aspect of the myriad of gait components. The lack of an observed relationship between these two variables does not mean that abnormal walking patterns do not place excess stress on the knee joint. Future research can investigate other aspects of self-selected walking gait.

The relationship between upper leg muscle flexibility and gait is complex and multi-joint. Furthermore, there is little data examining how muscle flexibility affects gait in healthy individuals. This makes it difficult to draw conclusions from the available data. Additionally, muscle stiffness itself may not be pathologic, but the collected data indicates an association between flexibility and gait patterns in this cohort. Therefore, chronic thigh muscle stiffness may lead to lasting effects on gait. If these changes in gait cause chronic increased stress on the knee joint, OA may progress more quickly secondary to upper leg muscle rigidity. Future analyses will use other aspects of self-selected walking gait to observe patterns in the relationship between upper leg muscle flexibility and gait.

Our current study has some limitations. A higher BMI may have decreased the average and peak amplitude of the AEs from the knee joint due to increased subcutaneous fat thickness between the knee joint and the surface microphone probe. Future studies will incorporate waveform analyses

to characterize the acoustic emissions recorded, as other studies have done (Yiallourides et al., 2021). Waveforms may not be impacted by changes in BMI. Furthermore, knee AEs paint an incomplete picture, in that the knee joint cannot be visualized with this method. Future work could utilize imaging technology, such as ultrasound or magnetic resonance imaging, to better understand the state of the knee joint.

CONCLUSION

In the cohort studied, HQ ratio is not significantly related to biomarkers of knee health, including knee acoustic emissions, and heel strike angle is not significantly related to acoustic emissions from the knee joint. However, we found that an individual's HQ ratio may provide insight into their overall health. This is highlighted by the negative relationship between HQ ratio and BMI. The mechanism to explain this relationship remains unknown. Increased hamstring stiffness shows a significant relationship with decreased heel strike angle on the left leg, which was more frequently reported as the nondominant leg. We found that increased right quadricep flexibility correlates with increased right heel strike angle. Therefore, differential levels of upper leg muscle flexibility may contribute to changes in gait. Future work must be done to determine whether these gait changes increase the stress placed on the knee joint during walking.

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Isometric Hamstrings:Quadriceps Strength Ratio, Flexibility, and Gait Pattern as Predictors of Knee Health

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Antecedents of Procrastination: Examining the Role of Academic Identity and Self-Esteem

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ABSTRACT

Procrastination is a widespread problem among students, yet feasible solutions have remained elusive. Recognizing the detrimental impact of procrastination on students' potential, this study aims to investigate the underlying factors contributing to this problem. Specifically, this study explores the associations between academic identity, self-esteem, and procrastination in both male and female participants. Previous research suggests a negative correlation between self-esteem and procrastination. There has been substantially less research examining the relationship between academic identity types (i.e., achieved, foreclosed, moratorium, and diffused academic identity) and procrastination. The current study surveyed 244 college students about their personality habits, academic identity, and self-esteem. It was hypothesized that self-esteem would moderate the relationship between achieved academic identity and procrastination. Multiple regression was used to test the relationship between multiple theorized predictors of procrastination, specifically, self-esteem, achieved academic identity, and self-reported procrastination. Regression analysis supports the hypotheses. Specifically, there was a significant interaction effect between self-esteem and achieved academic identity. Regression results suggest self-esteem is more predictive of procrastination when achieved academic identity is low. Additionally, results suggest students who have not made a commitment to academic goals and values are more likely to procrastinate. Theoretical implications for studying academic identity and procrastination will be discussed, along with intervention recommendations for college students.

KEYWORDS: academic identity, self-esteem, procrastination

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Antecedents of Procrastination: Examining the Role of Academic Identity and Self-Esteem

INTRODUCTION

Procrastination, delaying or postponing an important task, is a prevalent problem among college students. There are varying definitions surrounding the action of procrastination. The most common definition, and one we will be using in this paper is to "voluntarily delay an intended course of action despite expecting to be worse off for the delay" (Steel, 2007, p. 66). Procrastination is an issue that impacts 70-95% of students, making it a stumbling block for far too many college students (Steel, 2007). This issue is significant enough that it may increase stress and anxiety, resulting in lowered academic performances, lower grades, loss of scholarship, and academic suspension/probation (Korstange et al., 2019). Considering these factors of procrastination, this study intends to investigate two antecedents of procrastination: academic identity and self-esteem in college students.

Procrastination has been investigated as a personality trait. This means that procrastination can be a habitual part of someone's personality and can bleed into their academic life. In the Big Five Factors Personality, studies show that procrastination is largely related to a lack of conscientiousness (Schouwenburg & Lay, 1995). Lack of conscientiousness is often described as a lack of goal-setting, planning, and responsibility. The association between procrastination and conscientiousness should not be overlooked; goal setting would be a variable associated with someone's sense of achieved academic identity. Previous literature shows a positive correlation between achieved academic identity and consciousness (Burbidge et al., 2018), which is rational as achieved academic identity is characterized by commitment and planning towards specific academic goals. Personality traits are predictive of adaptive and maladaptive outcomes and can be adaptable. Previous literature shows that personality traits change, especially among the age of young adulthood (20-40 years; Roberts & Mroczek, 2008). Ultimately, personality traits are malleable and can be improved, most notably for the age group we are studying; therefore, exploring the direct relationship between procrastination and academic identity is crucial as literature on these variables is currently missing.

Self-Esteem

Low self-esteem is another personality trait that is highly predictive of procrastination (Yang et al., 2021). Low self-esteem is characterized by feelings of fear of failure that contribute to the inability to initiate action to avoid negative implications of failure (Yang et al., 2021). Previous literature shows that individuals with low self-esteem tend to have a negative opinion of themselves, and self-esteem is a key characteristic of habitual procrastination (Steel, 2007). Importantly, the literature consistently suggests a negative relationship between procrastination and self-esteem. The relationship between self-esteem and procrastination has also been shown to be mediated by other factors such as fear of failure.

There are different theoretical perspectives used to examine procrastination and self-esteem. From the cognitive psychology perspective, procrastination is related to low self-worth (Steinert et al., 2021). Specifically, procrastination allows people to discount failure to their procrastination instead of their abilities, helping them avoid feelings of personal failure. Individuals with low self-worth tend to have low expectations for their performance, and delaying the task helps to avoid feelings of inadequacy. Procrastination is also studied from the psychodynamic perspective, which examines subconscious feelings, unresolved conflicts, and opposing needs (Steinert et al., 2021). Self-esteem is a predictor of procrastination as it is a protective layer of self-worth (Topalsan, 2020). Ultimately, self-esteem is a key factor that is directly and indirectly related to procrastination. Thus, understanding it is essential in finding interventions

Antecedents of Procrastination: Examining the Role of Academic Identity and Self-Esteem

for the problem. This study will examine self-esteem as a moderating variable between achieved academic identity, and procrastination.

Academic Identity

Academic identity is defined as the perception of an individual's experiences in the two dimensions of exploration and commitment (Was & Isaacson, 2008; Was et al., 2009). Academic identity is classified in four different facets based on exploration and commitment: diffused, moratorium, foreclosed, and achieved. These facets of academic identity were proposed by Was and Isaacson (2009) derived from Marcia's Theory of Identity Status. An individual can be high, low, or average in any of the facets. Someone that has a diffuse academic identity lacks both exploration and commitment in their academic career. For example, an individual who is in college with an undeclared major might lack the effort to explore and/or commit to any major. In contrast, an individual in moratorium would be in a stage of exploration but still lacking any firm academic commitments. A student high in moratorium may be exploring multiple majors but not committing to any. A person with a foreclosed identity is fully committed to their academics due to external factors (e.g., family, social pressure) but lacks personal exploration. For instance, a student with a foreclosed identity might choose biology without exploring other avenues because their family told them to. Lastly, a person with an achieved academic identity has fully explored and made firm commitments towards their academic goals. This would be someone who has explored potential majors and commits to one due to personal interests (Was & Isaacson, 2008; Was et al., 2009).

Currently there is a need for more literature regarding academic identity and its role in procrastination. A study by Sharifi and Ashouri (2022) reported a strong negative correlation between academic identity and procrastination. Therefore, someone with a high academic identity was less likely to be associated with high procrastination. In contrast, someone who scores high on the diffused academic scale was more likely to be associated with high procrastination, likely because it is characterized by low commitment and exploration. This suggests that students with low academic identity are more likely to engage in self-handicapping behaviors like procrastination (Chorba et al., 2012). The current study used data collected from 244 undergraduate students regarding procrastination, academic identity, and self-esteem to test the stated hypotheses.

Based on previous research by Chorba et al., (2012) suggesting a positive relationship between academic identity and procrastination, we hypothesize that:

Hypotheses 1a: Diffused academic identity will have a significant positive correlation with procrastination.

Hypothesis 1b: Moratorium academic identity will have a significant positive correlation with procrastination.

Hypothesis 1c: Foreclosed academic identity will have a significant positive correlation with procrastination.

Hypothesis 1d: Self-esteem will have a significant negative correlation with procrastination.

Hypothesis 1e: Achieved academic identity will have a significant negative correlation with procrastination.

Hypothesis 2: Self-esteem will significantly moderate the relationship between procrastination and achieved academic identity.

METHODS

Participants

Participants consisted of 244 students recruited using the SONA pool from a Southern California University. Students participated for course credit. The participants could pick any study to participate in a pool of available

Antecedents of Procrastination: Examining the Role of Academic Identity and Self-Esteem

studies in the SONA participation system or write short essays to satisfy the course credit. IRB approved the current study before data collection. The study comprised 97 males, 145 females, and 2 participants identified as non-binary or other. The mean age of participants was 19.7 (SD = 1.53). The sample was diverse, with 40.98% Asian or Pacific Islander, 38.93% Latinx/Hispanic, 7.37% Multiracial, 4.51% White, 4.09% Middle Eastern, 2.04% African American, 1.23% other, and 0.82% Native American.

Design

The design of this study is correlational. We utilized validated survey measures, specifically the PPS (Steel, 2010), AIM (Was et al., 2009), and RSES (Rosenberg, 1965) to measure procrastination, academic identity, and self-esteem. Measuring these variables allowed us to test our hypotheses and determine the relationships between the variables. By utilizing RStudio for hypothesis testing, we conducted a Pearson correlation analysis, a regression analysis, and tested for interaction effects between the variables of procrastination, self-esteem, and academic identity.

Measures

Procrastination. Procrastination was measured using the Pure Procrastination Scale (PPS; Steel 2010). The PPS consisted of 12 items with no reverse-coded items and was measured on a Likert scale, ranging from 1 (strongly disagree) to 6 (strongly agree). An example item includes "I delay things beyond what is reasonable." (α = .90). The alpha coefficient allows us to evaluate the reliability of the scale, and an alpha of .90 suggests the scale is reliable. Alpha coefficients are calculated by dividing the scale's average covariance by its average total variance, and all alpha coefficients were calculated using RStudio, a statistical software.

Academic identity. Academic identity was measured using the Academic Identity Scale (AIM; Was et al., 2009), consisting of 40 items divided between 4 sub-scales. These sub-scales are diffused, moratorium, foreclosed, and achieved. The AIM was measured on a Likert scale from 1 (Not at all like me) to 5 (Very much like me). Example items include "My priorities in school are in transition. Some days, I am serious; other days, I have other priorities." (Moratorium subscale; α = .84); "I've never decided on my own about college. I just did what friends and family expected of me." (Foreclosed subscale; α = .78); "A college education is a high priority for me, and I'm willing to make sacrifices." (Achieved subscale; α = .75); and "Sometimes I think the reason I'm in college is I have nothing better to do." (Diffused subscale; α = .80). No items were reverse coded.

Self-esteem. Self-esteem was measured using the Rosenberg Self-Esteem Scale (RSES; Rosenberg; 1965). The RSES consisted of 10 items, with five items being reverse-coded. The ranking was calculated on a Likert scale of 1 (Strongly Disagree) to 4 (Strongly Agree). An example item includes "On the whole, I am satisfied with myself." (α = .89).

Procedure

Participants were recruited from the SONA participation pool, and they participated for two research credits. Participants could choose which study they wanted to participate in from a pool of available ongoing studies. All survey measures were administered online via Qualtrics. Participants that signed up for this study received an email with a Qualtrics survey link and instructions to complete the survey measures in one continuous session. Researchers informed participants that the study was about academic experiences. Participants completed the survey measures within two days of receiving the emailed link and instructions. A power analysis was conducted in G*Power 3.1.9.7 (Faul et al., 2007) and results suggest a minimum sample size of 281 is adequate to find a moderate effect size between predictors (i.e., self-esteem and academic

Antecedents of Procrastination: Examining the Role of Academic Identity and Self-Esteem

identity) and outcome (i.e., procrastination). Given our sample size of 244, we conclude this sample has adequate power to find an effect.

RESULTS

Hypothesis 1a-1e

Hypothesis 1a-1e predicted procrastination would have a positive relationship with diffused (1a), moratorium (1b), and foreclosed (1c) academic identities; and a negative relationship with self-esteem (1d) and achieved academic identity (1e). Results suggest procrastination had a significant positive correlation with diffused (r = 0.48, p > .001), moratorium (r = .55, p > .001), and foreclosed academic identity (r = .22, p > .001). As hypothesized, procrastination showed a significant negative correlation with self-esteem (r = -.42, p > .001) and achieved academic identity (r = -.35, p > .001). To summarize these results, those who rated themselves higher on diffused, moratorium, and foreclosed academic identities were more likely to report procrastinating, while those who rated themselves higher on achieved academic identity and self-esteem were less likely to report procrastinating. These results are consistent with our hypothesis.

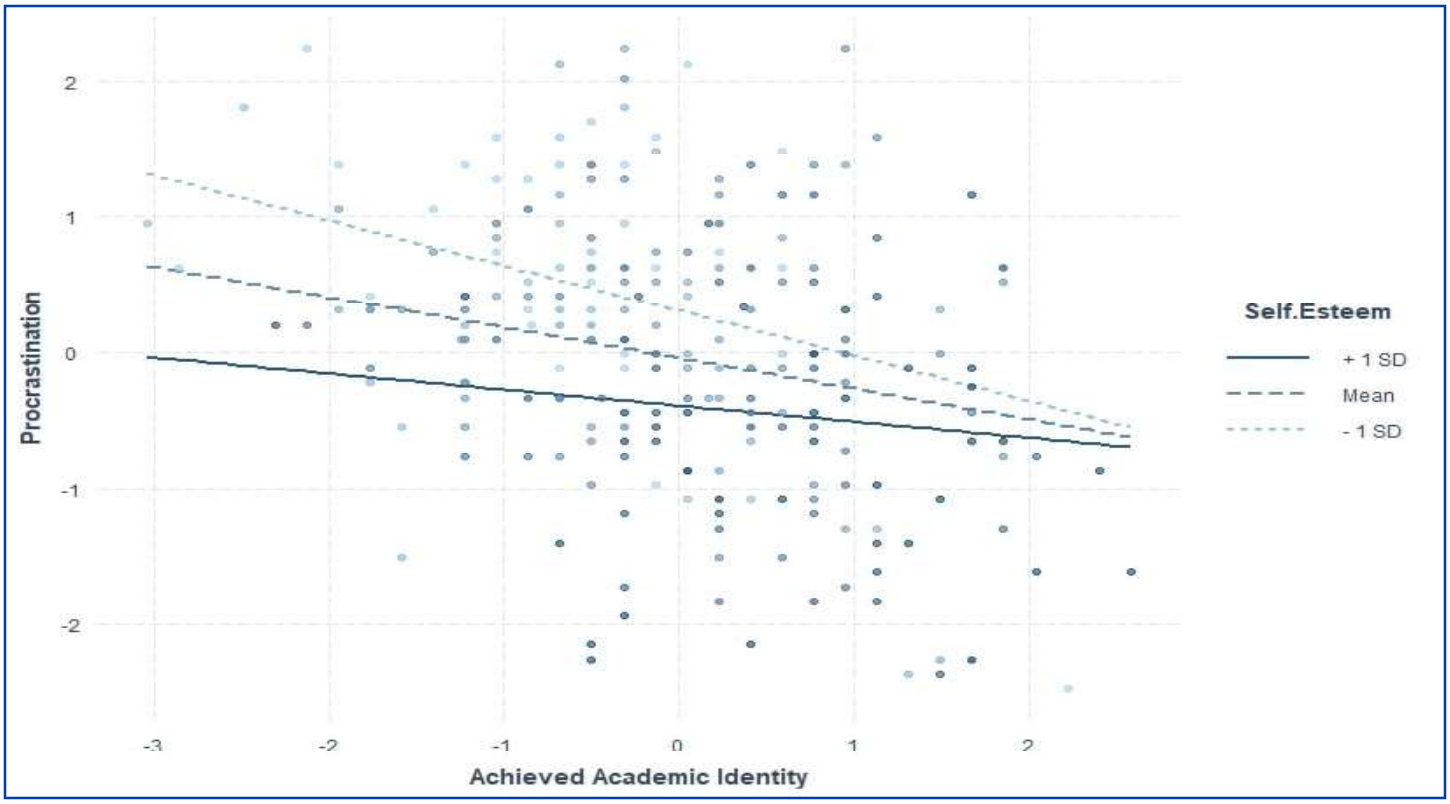

Figure 1. Self-Esteem's Moderating Role Between Achieved Academic Identity and Procrastination

Note. The dark blue line (bottom line) represents students high in self-esteem, the blue line (middle line) represents students average in self-esteem, and light blue line (top line) represents students low in self-esteem. The dots indicate scatter points of participant responses.

Hypothesis 2

Hypothesis 2 predicted that self-esteem would moderate the relationship between achieved academic identity and the outcome variable procrastination. Our data revealed a significant main effect of self-esteem (β (240) = -0.35, $p < 0.001$), a significant main effect of achieved academic identity (β (240) = -0.22, $p < 0.001$), and a significant interaction effect between self-esteem and achieved academic identity (β (240) = 0.11, $p = 0.041$). The graph was obtained by plotting the interaction between self-esteem and achieved academic identity (see Figure 1) with procrastination as the outcome variable. All graphing was done in RStudio. The graph suggests low self-esteem (denoted by -1 SD in the graph) is predictive of more procrastination when achieved academic identity is also low. Additionally, the graph suggests high academic identity is predictive of less procrastination for all levels of self-esteem (see the right side of Figure 1, where self-esteem lines converge).

DISCUSSION

Previous research regarding the relationship between procrastination and academic identity shows a negative correlation. The results of this study suggest that those higher in academic identity are less likely to procrastinate in academic settings, and this relationship is moderated by self-esteem. Specifically, those high in achieved academic identity and low in self-esteem predicted a low likelihood of procrastination. In contrast, those low in both academic identity and self-esteem predicted a high likelihood of procrastination. Additionally, results from this study suggest having diffused, moratorium, and foreclosed academic identities are predictive of more procrastination.

The conclusions of this paper may lead to a new perspective on how academic procrastination is examined, which may change the type of interventions used to tackle procrastination. Previous literature credits fear of failure, perfectionism, low self-worth, and anxiety as common antecedents of procrastination. These findings are valid and valuable to the study of procrastination, but literature has neglected to investigate the role of identity and academic identity on procrastination. Our study suggests procrastination can manifest itself in students who lack firm commitment and planning in an academic setting. Our study also finds a moderating effect of self-esteem which suggests students with low self-esteem and lack of academic plans may need more attention and intervention to avoid procrastination.

This study offers a new perspective on antecedents of procrastination by using identity theory—Academic Identity Statuses derived from Marcia's theory of Identity—which is rarely discussed in psychology. Steel (2007) suggests that low self-esteem is one of the common traits related to procrastination because individuals with low self-esteem, or a negative view of oneself, also tend to have a fear of failure. Subsequently, this is related to the way individuals approach tasks. For instance, students with low self-esteem may fear doing poorly on an assignment and maladaptively cope with that fear by avoiding the assignment. Our study suggests self-esteem is not highly predictive of procrastination when academic identity is high, suggesting academic identity plays a valuable role in procrastination in college settings. Our study also suggests a lack of exploration and commitment (diffused identity) are shown to be highly predictive of more procrastination. Therefore, providing students with more outlets to explore their academic interests may resolve habitual procrastination in academic settings.

Sharifi and Ashouri (2022) suggest that students who lack a stable academic identity may become "too hot-tempered" and "nervous" when doing academic assignments, contributing to a lack of concentration.

This suggests that the lack of academic exploration and commitment may contribute to irrational behaviors that lead to procrastination; therefore, helping students actively explore and commit to their academic pursuits may aid in decreasing their procrastination.

The current study is a unique piece of literature that explores the relationship between academic identity and procrastination across all dimensions of academic identity. It is also the first to explore the relationship between achieved academic identity and procrastination moderated by self-esteem, informing our views of how procrastination and self-esteem are linked. Our study can help students become more aware of their academic identity and understand the benefits of exploring their academic identity. Additionally, our study consisted of a diverse population of students, capturing the experiences and attitudes of students from multiple different ethnic backgrounds.

LIMITATIONS AND FUTURE DIRECTIONS

The current study used survey data to explore the relationship between procrastination, academic identity, and self-esteem. Survey data is valid for such research because concepts like academic identity and self-esteem are best assessed by asking the participants. Additionally, all survey measures were validated, and alpha values for the scales are acceptable. Like many other studies, this study measured procrastination using the PPS scale, which is a validated survey measure. However, survey data is not ideal for concepts like procrastination, as it does not directly study the behavior in action. Therefore, future studies should strive to use behavioral measures of procrastination.

Viable future directions include studying how academic identity changes as students progress through college, utilizing a longitudinal research design. To the authors' knowledge, no such study has been published yet. This is an important aspect to consider because identity, like personality, is malleable and better understanding how it changes can help researchers make better recommendations to address procrastination in academic environments.

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A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

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ABSTRACT

The dynamics of epithelial cells during wound healing exhibit significant complexity, notably in their size-dependent behavior. This work aims to depict a fundamental mechanism underlying this size dependence in cellular dynamics by developing a computational model Our research question investigates how the physical size of epithelial cells influences their motility and behavior patterns, specifically during the epithelial-to-mesenchymal transition critical for wound healing. Thus, we propose a model where the key mechanism involves a field of spatially coupled forces acting on the cell membrane, driven by the dynamics of actin monomers. These monomers, randomly distributed within the cell, become focal points for membrane protrusions, thus influencing cell behavior. Our model succinctly captures the essence of size-dependent cellular dynamics without resorting to changes in gene expression patterns, offering new insights into the variations in cell behavior. Through this computational framework, we demonstrate that the diversity in cellular responses during wound healing can be fundamentally attributed to differences in cell size. The model's insights into the correlation between cell size and motility highlight how the physical properties of cells influence wound healing.

KEYWORDS: epithelial-to-mesenchymal transition, size-dependent dynamics, computational model, protrusions, computational software, wound-closure

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A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

INTRODUCTION

Biological background

Epithelial cells, commonly recognized as the building units of the body, play a role in establishing protective barriers [1]. They contribute significantly to maintaining tissue integrity and overall balance within the body. Epithelial cells serve as the building blocks for linings in organs and structures. Not only do they act as barriers against environmental hazards, but they also actively participate in numerous physiological processes. These processes include absorbing nutrients in the tract, facilitating gas exchange, and filtering substances in the renal system. Another role is their involvement in tissue and organ regeneration. In cases of injury or damage, these cells play a part in the body's response to restore tissue integrity.

This natural healing process is supported by a mechanism known as epithelial to mesenchymal transition (EMT), which signifies a shift from their usual stationary state to motile. Epithelial cells undergo EMT, enabling them to close wounds, including minor cuts on the skin's outer layer and more severe injuries. The intricate dance of cells during the healing process regulated by EMT remains an area of great scientific interest and exploration [1,2].

Wound healing requires epithelial cells to fill the gap caused by the wound as fast as possible. Besides simply proliferating, epithelial cells start exhibiting different behaviors depending on how close they are to the wound edge [3,4]. The cells on the edge experience dramatic stresses due to the difference between the wound interior and the opposite side filled by neighboring cells. Such cells tend to extend long protrusions (so-called lamellipodia) towards the wound center and become motile (through EMT) in the direction of their protrusions. These cells are regarded as "leaders." Cells, which are adhered to the leader, follow it and thus are called "followers." Leaders have a pancake-like shape; they are flat and occupy a much larger area than followers. It is unclear if leaders generate force and pull followers toward the wound center or if leaders and followers have the same level of motility and the leader just guides followers [5]. The entire cluster of leaders and followers migrates persistently in an appropriate direction to close the wound. The coexistence of leaders and followers is an example of when epithelial cells exhibit diversity in sizes, and the cell's dynamics strongly depend on its size: leaders, when not adhered to other cells, are almost stationary without significant variations in shapes, whereas individual followers oscillate and extend fast thin protrusions (so-called filopodia). It is worth noting that the proliferation rate of leader cells is much lower than follower cells [4].

The emergence of leaders and followers is associated with the specific setting of the tissue's front propagation during wound closure. However, other types of cellular configurations are of great importance. For example, when cells are at low confluency, they form small clusters or migrate individually at the early stages of tissue formation. In these cases, besides leaders and followers, individual cells of the intermediate area can be observed; that is, they are larger than followers but smaller than leaders [2]. Extensions for these cells are smaller than for followers, and shape variations are smooth. For example, when retracted, an extension turns into a new extension nearby, or an extension does not retract fully. Still, it starts moving along the cell boundary, thus showing a boundary traveling wave. Note that, on the one hand, all these cells—small followers, large leaders, and intermediate cells—originated from the same tissue, where all cells are densely packed and look the same [4].

On the other hand, in this hierarchy of cells, each "class" behaves differently and has a specific function in collective cell migration. Similar to how leaders' behavior acquires them a function of navigating the

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

repairing tissue, mechanical properties of intermediate cells may elucidate their role in collective cell migration.

Purpose of the Study

In this work, we aim to develop a theoretical framework via a computational model, providing a mechanical description of cells exhibiting different behaviors so that these behaviors depend on a single geometric parameter, such as the cell size. Conversely, all other parameters, including microscopic ones, remain across all cells. This uniformity ensures that any observed differences are due to the variable under investigation.

Typically, cell shape and motility dynamics are described as a subtle interplay between cell constituents, such as actin filaments, myosin motors, and adhesion proteins, as well as the elasticity of the cell membrane [6]. Motility models assume that actin filaments tend to grow (polymerize) at the leading edge by attaching actin monomers on their front, thus pushing the elastic membrane forward. We focus on monomers and polymers' actin dynamics to elucidate the size-dependent qualitative behavior. More precisely, we consider randomly distributed monomers along the cell body and assume that they tend to cause intensive actin polymerization and growth of an extension when the number of actin monomers near a boundary point exceeds a certain threshold. Finally, we note that epithelial cells transitioning to a motile state are located on the edge of a tissue or crawl individually. This work will focus on the dynamics of an individual cell that does not adhere to any other cell.

Relevance to Current Research

Our work is situated within the broader context of cell biology research, where understanding the mechanisms of cell movement is essential. By focusing on size dependence, this study addresses a gap in research that predominantly assumes that cell differentiation or external chemical cues can cause differences in the behavioral patterns of cells. Our computational model provides a complementary perspective, offering insights into the physical and mechanical aspects of cell motility that are influenced by size. This unique angle broadens the scope of current research and sets the wave for future investigations into how size and chemotaxis interact to govern cell behavior in complex biological processes.

METHODS

We employed a methodology encompassing Physics, Mathematics, and Computational Modeling principles to comprehend size-dependent dynamics for motile epithelial cells. In modeling the cell, we followed the concept of the Subcellular Element Method [7]. We consider a two-dimensional domain representing the apical view of a cell crawling on a substrate. The cell membrane is described by a finite sequence of nodes connected by elastic springs. These nodes with springs form a closed curve determining the cell's shape. Springs connect the neighboring nodes only; see Figure 1A. As time progresses, the cell maintains its initial area with minor fluctuations. This aspect of the model enables us to simulate cells of a specified area and investigate the impact of this area on the dynamics of cell shape. Further details on how we model cell shape and the mechanisms of cell motility through the polymerization of actin monomers are provided in the Methods section below.

Model of Cell Shape

For each node, we impose the force balance. Namely, the viscous drag force is balanced by the spring force, surface tension, area preservation force, and protrusion force. The balance equation results in an ordinary differential equation for each node, and the solution of this equation, if protrusion force is neglected, tends to minimize the elastic energy of each spring, surface tension energy, and the discrepancy between the current

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

area of the cell and the initial one:

$$r_i'(t) = -\nabla_{r_i}(\mathcal{E}_{spring} + \mathcal{E}_{s.tension} + \mathcal{E}_{area}) + F_{protr}. \quad (1)$$

Here, $\mathcal{E}_{spring} = k_{spring}(|r_i(t) - r_{i1}(t)| - L_0)^2 + k_{spring}(|r_i(t) - r_{i2}(t)| - L_0)^2$ is the energy of two springs from both sides of the node r_i (indexes for two neighboring nodes are denoted by $i1$ and $i2$, see Figure 1A) and L_0 is the equilibrium spring length. The number of nodes is denoted by N_{mem}. The surface energy is defined as $\mathcal{E}_{s.tension} = k_{tension}\sum_{i=1}^{Nmem}\cos(\varphi_i)$ and φ_i is the angle formed by the two springs at the node r_i, and the area preservation energy is given by $\mathcal{E}_{area} = k_{area}(A(t) - A_0)^2$, where $A(t)$ is the cell area at time t and A_0 is the initial area of the cell. Model parameters k_{spring}, $k_{tension}$, k_{area}, L_0, and A_0, are calibrated so that numerical simulations are stable and observable dynamics is biologically reasonable. The force F_{protr} comes from protrusion, as described below.

When protrusive forces are disregarded, $F_{protr} = 0$, the minimization of total cellular energy leads to the convergence of the cell shape toward its equilibrium state, which is a circle. Specifically, the energy term $\mathcal{E}_{spring}$ penalizes deviations in the cell shape when the distance between adjacent nodes differs from the equilibrium spring length L_0. By minimizing the energy, $\mathcal{E}_{s.tension}$, the cell membrane tends to rectify at each node. Given that the membrane is a closed curve, a circular shape minimizes the energy term $\mathcal{E}_{s.tension}$. Additionally, the third energy term in equation (1), $\mathcal{E}_{area}$, acts to maintain the cell's initial area.

The dynamics described by equation (1) emerge from the interplay among these three energy minimization processes. For instance, if there is a disparity between the radii of the circle with the perimeter $N_{mem} \cdot L_0$, favored by the minimization of $\mathcal{E}_{spring}$, and the circle with the area A_0, favored by $\mathcal{E}_{area}$, the actual radius is determined by the coefficients k_{spring} and k_{area}. For example, when $k_{spring} >> k_{area}$, the minimization of $\mathcal{E}_{spring}$ dominates the minimization of $\mathcal{E}_{area}$, and the equilibrium radius is close to $(2\pi)^{-1}N_{mem}L_0$.

Model of Protrusions

We model cell protrusions extended due to an internal activity as follows. We track individual free actin monomers inside the cell (see green dots in Figure 1B). These monomers must be understood as coarse-grained since the number of monomers exceeds the one we use in the model by several orders of magnitude. A protrusion is formed if monomers are randomly concentrated at a membrane node (see Figure 1B). Namely, denote the number of monomers about r_i by $N_{m,i}$. We call corresponding monomers adjacent to the membrane node r_i (depicted as black dots in Figures 1B and 1C). Then, the condition for the formation of a protrusion r_i is $N_{m,i} > N_{threshold}$, where $N_{threshold}$ is the threshold value for the number of adjacent monomers. The formation of protrusion is incorporated in the modeling equations (1) via the term F_{protr}, which is non-zero when the condition $N_{m,i} > N_{threshold}$ is satisfied. The force F_{protr} is directed along the outward normal, and its magnitude and duration time values are chosen to resemble biologically relevant behavior. In addition, we model that adjacent monomers are attracted by the corresponding membrane node, preventing them from participating in protrusions elsewhere. When the protrusion is retracted, all monomers are pushed back inside the cell. All monomers not engaged in a protrusion exhibit a random walk confined by the polygon generated by membrane nodes. The formation of protrusions, represented by the term F_{protr} in Equation (1), serves as an active component of the system, actively maintaining the cell in a state away from equilibrium.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

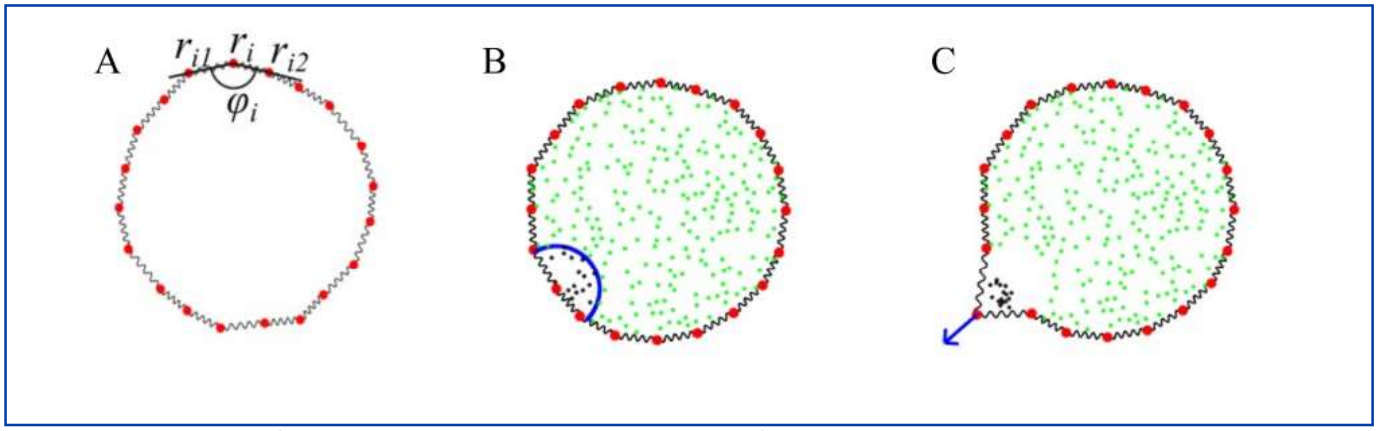

Figure 1. Illustration of the model setup. (A). Representation of cell shape as a polygon whose vertices are membrane nodes. Each side of the polygon is modeled as a spring and depicted as a zig-zag-shaped segment. If no Fprotr is exerted, Equations (1) result in convergence of the polygon to the regular one as time evolves, and, if the number of membrane nodes is very large, to a circle (B). Representation of cell with monomers (black and green isolated dots) and no protrusions. Each time step, the number of monomers at the vicinity of a membrane node is measured; the boundary of the vicinity is indicated as a blue arc and the corresponding monomers are black isolated dots (C).Illustration of protrusion formation, blue arrow depicts the force F_{protr}.

Implementation of the Computational Model

The model is developed using MATLAB (code is 367 lines). Ordinary differential equations in (1) are solved with the Forward Euler Method with a sufficiently small time step. The area of the cell $A(t)$ is calculated using the shoelace formula. The Brownian dynamics of the monomers are modeled as random jumps with standard deviation, $\sqrt{2D \cdot dt}$) for each time step dt, with the condition that a monomer does not exit the cell's boundary during these movements. As noted earlier, the values of the model parameters are carefully selected to ensure the stability of the computational code and to accurately reflect dynamics observed in various experimental and theoretical studies [2,6,7]. These values can be further calibrated using experimental data.

RESULTS

We numerically simulated cells of three different sizes. Specifically, we initialized cells as circles so that membrane nodes form a regular polygon, and we consider radii R=1.0, R=1.2, and R=1.6 corresponding to small, intermediate, and large cells, respectively. At the same time, we keep the number of membrane nodes N_{mem} and the number of free monomers N_{mon} the same for all radii, we adjust the spring equilibrium lengths according to the following equation:

$$L_0 = 2R \sin(2\pi/N_{mem}) . \quad (2)$$

Formula (2) is derived from representing the cell as a regular polygon with N_{mem} vertices so that R is the radius of the circumcircle of the polygon and L_0 is the side length. Monomers are generated randomly and uniformly inside the cell.

Representative cell dynamics obtained from the results of numerical simulations are presented in Figure 2. The small cell (R=1.0, see the first row in Figure 2) exhibited single or multiple protrusions at various distances from each other. When a protrusion is retracted, the next one may extend at a random point along the membrane. As we increase cell radius R and consider an intermediate cell (R=1.2, see the second row in Figure 2), correlations in protrusion locations become more evident. For example, the cell in the second row in Figure 2 extends three protrusions side-by-side in the

presented images. However, when we consider large cells (R=1.6, see the third row of Figure 2), protrusions are less frequent; they are usually single at each given frame and extend at random membrane locations. The decrease in correlations is because these correlations do not have time to preserve before the subsequent protrusion starts extending. Since the initial locations of monomers and their dynamics are random, we simulated the model with many realizations of initial conditions. The qualitative behavior was consistent throughout the realizations and the description of Figure 2 above.

Statistical properties of cell dynamics were also investigated. First, the probability of having a protrusion (or, equivalently, the protrusion frequency) decreases as we increase the cell size, see Figure 3A. It can be explained by that the number of monomers N_{mon} is the same for all cell sizes, implying that a larger cell will have a lower monomer density and, thus, a smaller number of protrusions. Similarly, the number of monomers protruding the membrane decreases with the cell size, see Figure 3B. Note that, though the protrusion frequency and the number of engaged monomers are strongly related, these two quantities have no one-to-one relation. To study the spatial distribution of protrusions, we computed the average cosine of the angle between two consecutive protrusions, $[\cos \Delta\varphi_{protr}]$. This quantity is close to 1 if protrusions tend to appear next to each other, and this quantity is close to -1 if protrusions appear on opposite sides of the cell. We see in Figure 3C that dependence on cell size is not monotonic: $[\cos(\Delta\varphi_{protr})]$ is positive for R=1.0, and then it becomes negative for R=1.2, slightly increasing for R=1.6. This observation supports the idea that even in a simple mechanical model, the behavioral patterns of a cell (and thus its role in cell migration) may be significantly altered by the change in a macroscopic parameter such as cell size. However, further research is necessary to uncover the correlations between successive protrusions and how these correlations depend on cell size. It is conceivable that there exists an optimal radius, R, with other parameters held constant, at which these correlations are maximized, leading to protrusions that emerge adjacent to each other, $[\cos(\Delta\varphi_{protr})] \approx 1$. Indeed, small cells with $R \ll 1$ tend to exhibit numerous protrusions, resulting in weaker correlations between protrusion locations. Conversely, in large cells with $R \gg 1$, the coupling between protrusions is diminished. This weakening of coupling is due to the constant number of monomers spread over a larger area, thereby reducing their concentration and, consequently, the correlations among protrusions.

Finally, the mean square displacement of the cell center was computed, see Figure 4. It was confirmed that a smaller cell moves faster than a larger one, similar to how smaller followers move faster than larger individual leaders [4].

In summary, the model demonstrates that smaller cells are more dynamic, exhibiting numerous protrusions and tending to displace their centers to a greater extent compared to larger cells. In contrast, larger cells remain closer to their equilibrium state, displaying minimal protrusion dynamics. This observed behavior aligns with the dynamics of isolated leader and follower cells in Madin-Darby Canine Kidney cells [3,4,5] which inspired this research.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

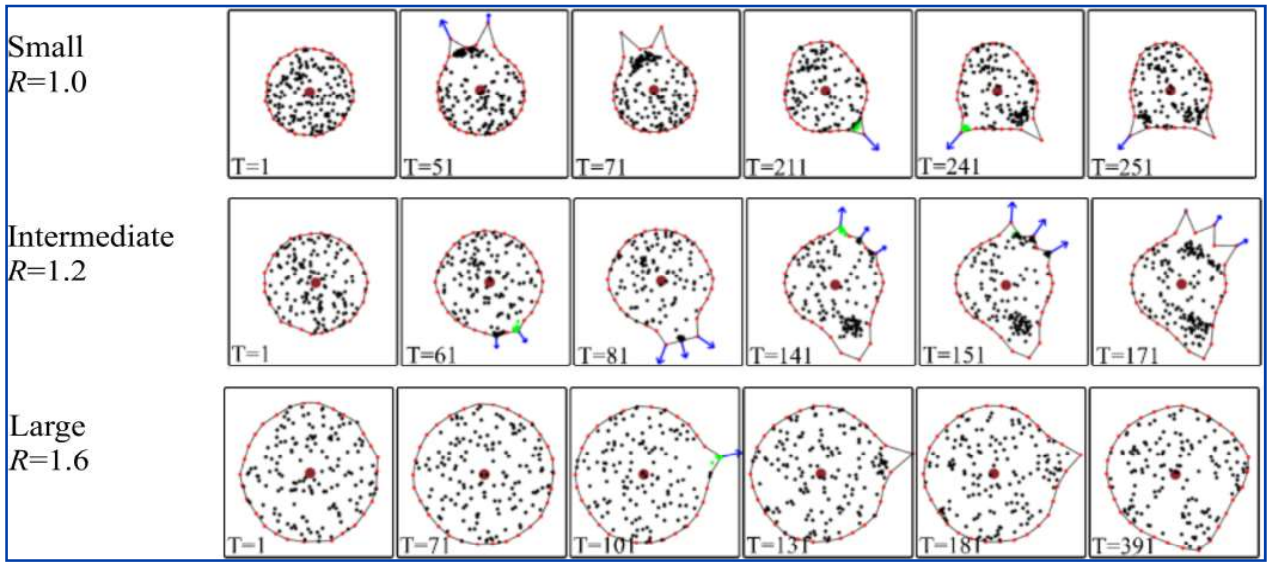

Figure 2. Cell dynamics obtained from numerical simulations for various values of radius *R*. Six subfigures in each row present the cell at different time instances (indicated in subtitles). Black and green dots represent free and engaged monomers, respectively. Red dots depict membrane nodes, and they are linked by black segments representing the cell boundary. Purple arrows are the protrusion active force. The large red dot represents the geometric center of the cell.

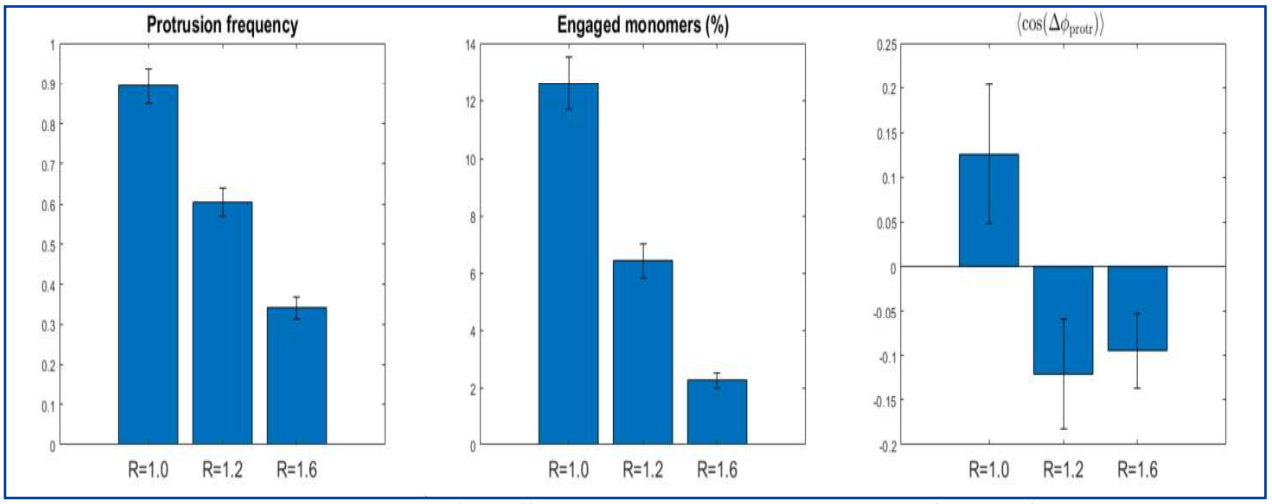

Figure 3. CBar graphs on cell activity for different sizes. Simulations were performed for 500 cells. (A). Protrusion frequency is defined as the number of existing protrusions per a time step. (B). Number of monomers engaged in protruding membrane (depicted in Figures 2, 3, and 4 as green dots). (C). The average cosine of the angle between two consecutive protrusions. The angle is computed with respect to the cell center.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

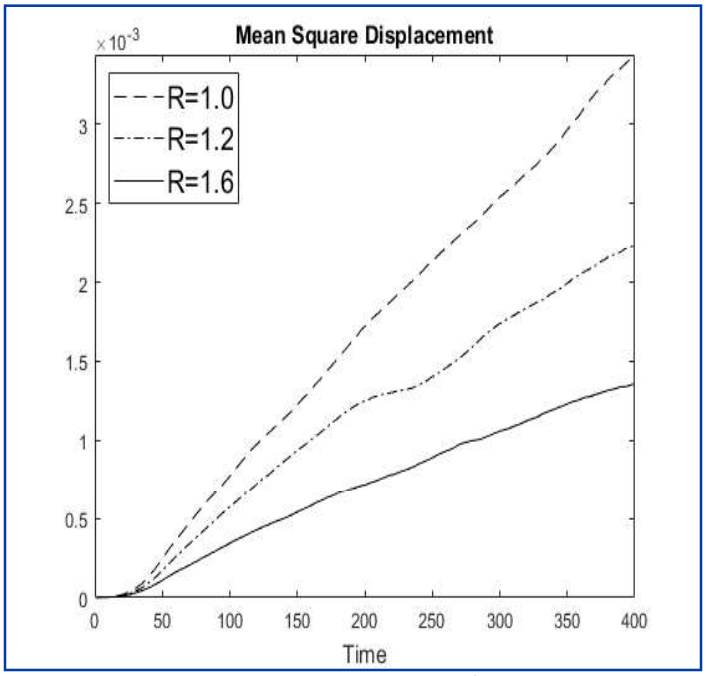

Figure 4. Mean square displacement of the cell center as a function of time. Three graphs correspond to various values of the cell radius, as indicated in the legend.

DISCUSSION

Cell motility results from very complex processes, including actin network formation, myosin dynamics, microtubule reorganization, and binding of adhesion bonds with the extracellular environment [8]. Additionally, a cell may alter its behavioral patterns in response to external chemical or mechanical cues [9,10]. However, cells may exhibit various behaviors even when no external perturbation is present, like leader and follower epithelial cells where isolated leaders are large (compared to follower cells) and passive and followers are active with a higher division rate [4]. It is known that epithelial cells, even within a single cluster, may be significantly different in size [11]. In this work, we hypothesized that the cell size may impact the geometric cell behavior. We confirmed this hypothesis by employing computational modeling. Our model utilizes actin network polymerization with freely floating monomers as the primary mechanism of protrusion formation in the spirit of a well-established modeling approach for actomyosin-driven cell motility [6,12]. Monomers engaged in actin network formation generate random forces on the cell boundary in our model. In this project, we assumed that these forces come from the dynamics of free actin monomers. Other intracellular processes may contribute to generating similar force fields, either related to the dynamics of myosin motors or cell-substrate adhesion. However, regardless of the biological origin of the force field, we believe that the size-dependent behavior is observed under two necessary conditions on the force field: the size-independent magnitude and spatial coupling. Here, the former condition is implemented by imposing that the number of monomers is constant throughout all sizes, and the latter one, spatial coupling, is through redistribution of monomer density, which deviates from the initial uniform configuration as time evolves.

Our model does not capture the rotation or traveling waves of intermediate-size cells observed in experiments and detailed computational models [13]. This is due to the symmetry of protrusions in the current model. As a future direction, we plan to incorporate a symmetry-breaking mechanism in the dynamics of an extension, which will allow for a preferred direction, clockwise or counterclockwise, and propagation of traveling waves along the cell's boundary. Finally, we plan to simulate the dynamics of heterogeneous cell clusters, where cells have different behaviors, and this difference originates from different cell sizes. Heterogeneity may benefit cell migration (thus wound closure) since different cells may acquire different functions useful for cell cluster navigation, chemotaxis, signaling, and cell activation [4].

The implications of our research are twofold. Firstly, it underscores the potential of intracellular processes beyond actin monomer dynamics to describe cell motility through similar force fields. It opens avenues for exploring the roles of myosin motors and cell-

substrate adhesion in future models. Secondly, our work highlights the necessity of incorporating mechanisms to break symmetry in extension dynamics, enabling the simulation of more complex behaviors like cell rotation and traveling wave propagation.

ACKNOWLEDGMENTS

I extend my heartfelt gratitude to Professor Mykhailo Potomkin from the Department of Mathematics at the University of Riverside for his invaluable support and guidance throughout this research. His expertise and encouragement have been instrumental to my work. Thank you, Professor Potomkin, for your unwavering dedication.

A Computational Model for Individual Epithelial Cells Captures How Shape Dynamics Depend on Cell Size

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A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

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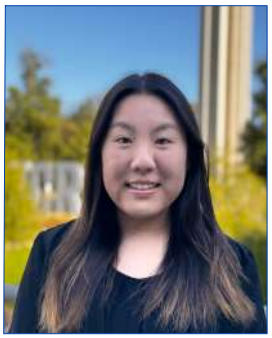
ABSTRACT

Influenza is one of the most prevalent viruses that has plagued millions worldwide. Every year health organizations encourage the public to get their flu vaccines to combat the flu season. Although the flu vaccines and medicines are widely available, it is estimated that flu viruses still cause 300,000-500,000 deaths every year. The two most common influenza virus strains are influenza A and B viruses, or IAV and IBV, respectively. It has been discovered that drug resistance develops very soon after a new drug is launched. It is highly demanded that anti-flu virus drugs with novel mechanisms be developed. Our lab has discovered that SUMOylation, a post-translational modification, is essential to the viral IAV and IBV life cycle. In this study, we have screened all the E3 ligases in the human genome to discover the SUMO E3 ligase responsible for the essential SUMOylation of IAV M1 protein using our Quantitative Fluorescence Energy Transfer(qFRET). We first determined the FRET spectrum of all E3 ligases with M1 protein and then quantified the FRET signals to provide a first-line examination of interactions. We then determined the E3-M1 interaction affinities, K_D, to ensure the real interactions. We found the E3 ligase PIAS1 has the highest affinity to M1 among other E3s. By understanding the interaction affinity between IAV M1 protein with SUMOylation E3 ligase, we hope to block the interaction between the PIAS1-M1 for novel anti-flu medicine development.

KEYWORDS: SUMOylation, influenza, quantitative Förster resonance energy transfer, viral proteins, protein interaction

FACULTY MENTOR - Dr. Jiayu Liao, Department of Bioengineering

Dr. Liao's research focuses on the development of a novel quantitative FRET(qFRET) technology platform for both basic research, such as dissecting host–virus and Ubl E3-substrates interactions, and translational research, such as SUMOylation inhibitor for anti-virus and anti-cancer therapeutics.



NIKKI WONG

Nikki Wong is a fourth year Bioengineering major. Under the guidance of Dr. Liao, she has been researching the interaction between E3 ligases with IAV M1 protein for the past two years. Nikki is a member of the University Honors program and is a Undergraduate Education Minigrant recipient. After graduation, Nikki will be pursuing her Master's in Cellular and Molecular Engineering.

SHIFA MIRZA

Shifa Mirza is a fourth year Bioengineering major. For the past year, she has worked in Dr. Liao's lab, studying the interaction of E3 ligases with IAV M1 Protein. Shifa is also the head of public relations for the Biotechnology Organization of Riverside and a mentor for the Bioengineering Graduate Student Associate. After graduation, Shifa will be pursuing a Masters in Bioengineering, with a concentration in Cellular and Molecular Biology.

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

INTRODUCTION

Influenza, a highly contagious respiratory virus, has and continues to pose significant challenges to public health. Categorized into four distinct subtypes - A, B, C, and D - it is responsible for causing both seasonal epidemics and sporadic pandemics worldwide. Specifically, the Influenza A virus (IAV) is known to trigger widespread outbreaks, exemplified by notable historical events such as the Spanish Flu of 1918 and the pandemic of H1N1 in 2009. During these global pandemics, the virus spreads swiftly from its origin, infecting millions across different regions in successive waves throughout the year. The impact of influenza is substantial, with the World Health Organization (WHO) estimating approximately 1 billion infections, 3–5 million severe cases, and 300,000–500,000 deaths annually.[1] The severity of the illness is contingent on multiple factors, including the specific viral strain and the cytokine storms in human immune responses.

Despite the development of seasonal flu vaccines, their efficacy remains suboptimal, particularly against IAV variants, which can mutate quickly with limited pre-existing human immunity. Additionally, the cyclical occurrence of pandemic strains, which antigenically differ from existing types—emerging approximately every 10–50 years—underscores the persistent threat posed by the disease. While significant strides have been made in public health awareness and virus technology, influenza continues to evolve and develop drug resistance to existing therapeutics, highlighting the pressing need for further research.[2]

Understanding the molecular mechanisms underlying influenza infection requires elucidating human-virus interactions represented as protein-protein interactions, which are fundamental to numerous biochemical and physiological processes. However, studying these interactions faces significant challenges, as over 80% of proteins exist in complex formations rather than in isolation, and it is very difficult to express viral proteins in other systems, such as bacterial cells or mammalian cells, necessitating sophisticated technologies for analysis. Förster Resonance Energy Transfer (FRET) is a phenomenon in which two fluorophores with overlapped excitation and emission spectrums can transfer energy through dipole-dipole interaction when they are close enough. As depicted in Figure 1, when proteins are in close proximity of 1-10 nm, FRET occurs between the two fluorophores, and the FRET spectrum can be elucidated.[4,13]

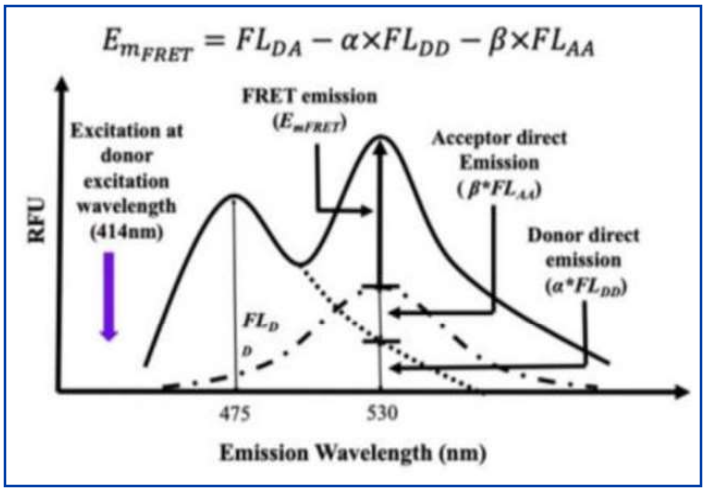


Figure 1: Fluorescent emission spectra explaining the energy transfer phenomenon between FRET donor and acceptor when the two fluorophores with overlapped excitation and emission spectrums are close to each other.[12]

Quantitative FRET (qFRET) technology emerges as a promising tool, as it is quicker, cheaper, and often less destructive to proteins than alternative assays, and it can be implemented as a high-throughput assay format. Specifically, qFRET can be used to assess protein interaction affinity (K_D), allowing further study into mitigating these interactions. This technology is developed explicitly towards post-translational modifications, such as SUMOylation, a pathway critical for the IAV M1 life cycle, as it utilizes the pathway for replication.[4] Using qFRET, we characterized the

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

interaction affinities of E3 ligase molecules within the SUMOylation pathway with the influenza IAV virus M1 protein. This research holds the potential to identify novel targets for antiviral drug development and deepen our understanding of the host-virus interactions, ultimately aiding in developing more effective strategies for combating influenza infections.

MATERIALS AND METHODS

Molecular Cloning of Constructs

pET28b plasmids encoding the fluorescent fusion protein, CyPet-TRAF6, PIAS3, PIAS4, RHES, hSTUB1, hRNF4, hMApl, parkin, PIAS1, hCBX4, hHDAC4, hHDAC4, hTRIM28, Dcst, Fbxw7, hCRBN, SOCS1, YPet-hSCNA, and YPet-M1, were cloned into *E.Coli* using varying strains of Electrocomp *E.Coli* cells via electroporation. Followed by a 1-hour recovery, Luria-Bertani (LB) agar plates with 50 µg/mL kanamycin were used to plate the transformed E.Coli cells. The following strains were used to amplify all the transformed plasmid DNA constructs followed by an extensive screening protocol to determine the highest strain value used for protein expression: BL21-CodonPlus (DE3)-RIL, ArticExpress(DE3)RP, BL21-CodonPlus, OverExpress™ C43(DE3), BL21(DE3), Shuffle®T7, BL21(DE3) pLysS, Rosetta (DE3)pLysS and OverExpress™ C41(DE3).

Protein Expression and Characterization of IAV M1 and E3 Ligases

The previously identified highest expressing strain was inoculated into a starting culture at 1:8 v/v of LB broth with 50 µg/mL Kanamycin, for resistance selectivity. Grown at a smaller culture overnight at 37°C and placed in a shaker at 250 RPM overnight, the culture was then transferred to 1 L of 2XYT media supplemented with 50 µg/mL Kanamycin and placed into a shaker at 250 RPM at 37°C until a proper optical density (O.D.) of 0.4-0.6 was reached at 600 nm absorbance. At the desired OD, protein expression was induced with 1M IPTG, a final concentration of 0.375 mM, and left to shake overnight at 16°C and 200 RPM.

After the induced culture was left to shake for 12-15 hours, the bacterial cells were collected by centrifugation at 4°C, 8000 xg for 5 minutes. The collected bacterial pellet was resuspended in centrifuge bottles physically, with the addition of 30 mL of Binding Buffer (20 mM Tris HCl, pH 7.4, 0.5 mM NaCl, 5 mM Imidazole). The resuspended bacterial pellet underwent sonication at ultrasonic frequencies to lyse the cells at alternating on and off phases of pulses for 7 minutes. Subsequently, the sonicated cells underwent 2 cycles of centrifugation at 4°C, 35,000 xg for 30 minutes, after which the supernatant was transferred into columns containing Ni^{2+}-NTA agarose beads while ensuring that pellet fibers were not included to prevent clogging. The attached protein and beads underwent two-column volumes of Wash Buffer 1 (20mM Tris HCl, pH 7.4, 300mM NaCl), two-column volumes of Wash Buffer 2 (20mM Tris HCl, pH 7.4, 1.5 M NaCl, 0.5% Triton-100), one-column volume of Wash Buffer 3 (20 mM Tris HCl, pH 7.4, 0.5M NaCl, 10mM Imidazole), and one-column volume of Interaction Buffer (150mM NaCl, 25mM Tris HCl pH 8, 5% glycerol) to strip unwanted bounded components to reduce non-specific binding.

After washing, proteins were eluted with 300 µL to 1mL 450 mM Elution Buffer (1 M Imidazole, Milliq Water) depending on the expected yield; 300 µL of Elution Buffer was allowed to flow through before collection. Dialysis Buffer (150 mM NaCl, 25 mM Tris HCl pH 8, 5% glycerol, DTT to a final concentration of 1 mM) was prepared in a glass beaker with a dialysis membrane bag prepared for each protein. Eluted proteins were pipetted into the dialysis bags and left to dialyze overnight at 4°C to remove excess salts.

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

Protein concentration was determined using the FlexStationI1384 to measure fluorescence intensities at Excitation 414 nm / Emission 475 nm (CyPet range) and Excitation 475 nm / Emission 530 nm (YPet Range). Purified protein underwent a 1:6 dilution before being pipetted into Greiner 384-well plates. The acquired fluorescence readings were calculated based on CyPet and YPet fluorescence standards to determine the concentration of the purified fluorescent-tagged proteins. Protein size was determined and confirmed via SDS gel electrophoresis; gel samples were prepared by taking 5 µg of the protein sample, 15 µL of SDS, and 15 µL of MilliQ H_2O. Samples were heated at 100°C for 5 minutes before loading into the polyacrylamide gel (Acrylamide, 10% APS, Temed, 1.5M Tris HCl pH 8.8, 1.5M Tris HCl pH 7.4, 10% SDS) with 3 µL of the DNA ladder. Electrophoresis was conducted at 100V for 3 hours; the gels were stained overnight with a Staining Buffer (Coomassie Blue R350, Methanol, Acetic Acid), then with a Destaining Buffer (Methanol, Acetic Acid) to better visualize the gel.

Em_{FRET} Assay

A 1µM:1µM CyPet and YPet fused with E3 Ligase and M1, respectively, assay was performed to generate preliminary interaction data. Varying excitation and emission peak wavelengths at 414 nm /475 nm and 475 nm/ 530 nm, were used for CyPet and YPet, respectively. When the fluorescent pair (CyPet and YPet) are placed in close contact at 2-10 nm with favorable orientations, then the excitation of the donor will excite the energy transfer from the acceptor. The coupling between the two fluorophores occurs due to the excitation of the donor, CyPet, which induces an energy transfer to the emission of the acceptor, YPet. As a result, it quenches the donor while exciting the acceptor. Preparing a 1 µM sample of CyPet and YPet with Interaction Buffer (150 mM NaCl, 25 mM Tris HCl pH 8, 5% glycerol) and 1 M DTT. Control samples were prepared with CyPet alone for the alpha value (α) and with YPet alone for the beta values (β); the ratio coefficient, α, is calculated to account for the emission peak of CyPet at 475 nm, as the ratio coefficient, β, is calculated to account for the emission peak of YPet at 530 nm. The prepared samples were pipetted in triplicate into 384 well plates.

Em_{FRET} was determined by utilizing Equation 1 to calculate true FRET emission.

Equation 1:

$$EmFRET = EmTotal - ((a * FLdonor) + (\beta * FLacceptor))$$

Spectrum reading is generated for qualitative interaction data collection. The following parameters are set for CyPet and YPet with 414 nm excitation, 455 nm cutoff and 475 nm excitation, 515 nm cutoff, respectively. For both samples, it was measured from 400 to 600 nm. Spectrum readings are to be performed separately for CyPet and YPet intensities.

K_D Determination

The dissociation constant (K_D) was determined by keeping the donor protein concentration at a constant of 0.1 µM and titrating the acceptor protein concentration from 0 µM to 25 µM. The fluorescent fusion protein pairs were combined into a total volume of 60 µL with Interaction Buffer (150mM NaCl, 25mM Tris HCl pH 8, 5% glycerol) and 1M DTT. Each titration was repeated in triplicates, again, to account for errors introduced due to pipetting variation. The prepared samples were incubated for 15 minutes in a 55°C water bath before being transferred to a Greiner 384-well plate. FlexStationII384 was used to measure fluorescence intensities at 414 nm /475 nm, 475 nm /530 nm, and 414 nm/ 530 nm. The settings necessary for the FlexStationII384 are selecting the "Endpoint" settings, setting the correct fluorescence intensities, and selecting the wells to be analyzed, and PMT constant gain at "Low" to allow for mixing.

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

Three wavelengths were recorded and the relationship between K_D and Em_{FRET} was determined by Equation 2.

Equation 2:

$$EmFRET * \left(\frac{[Acceptor]total - [Donor]total - KD + \sqrt{([Donor]total + KD - [Acceptor]total)^2 + 4 * KD * [Acceptor]total}}{[Donor]total + KD - [Acceptor]total + \sqrt{([Donor]total - [Acceptor]total + Kd)^2 + 4 * KD * [Acceptor]total}} \right)$$

Prism5 (GraphPad Software) was used to fit the Em_{FRET} values into Equation 2 to determine the K_D value. A non-linear regression fit was set with the donor concentration set at 0.1 uM and the initial K_D and $Em_{FRETmax}$ conditions set to zero.

RESULTS

The potential all human SUMOylation E3 ligases and as control, Ubiquitin E3 ligase, in human genome, TRAF6, PIAS3, PIAS4, RHES, hSTUB1, hRNF4, hMApl, parkin, PIAS1, hCBX4, hHDAC4, hHDAC4, hTRIM28, Dcst, Fbxw7, hCRBN, SOCS1, hSCNA, and IAV M1 genes were synthesized and cloned into pET28b vector with CyPet and YPet tag, respectively. Following an extensive screening process, strains exhibiting an expression ratio above 3 (induced vs. uninduced) were chosen to express proteins. Among these, hTRIM28 and PIAS1 were chosen for K_D determination because their FRET spectrum and EmFRET signals were positive for potential interaction. The proteins underwent purification through Ni-His affinity columns, and the eluted samples were collected for qFRET determination. An SDS gel electrophoresis was performed to confirm the fluorescent full-length proteins were maintained.

The FRET spectral analyses were conducted prior to determining the dissociation constant (K_D) for the interaction between the E3 ligase and the IAV M1 protein. The interaction spectra were examined at concentrations of 0.1 μM, 0.5 μM, and 1.0 μM/each protein, aiming to discern the specificity of the binding between the two proteins. In the initial investigation, CyPet-tagged E3 ligase at a concentration of 0.1 μM was engaged with YPet-tagged IAV M1 protein (Figure 2a-f). Subsequently, a parallel study was conducted wherein both CyPet-tagged E3 ligase and YPet-tagged IAV M1 protein were present at concentrations of 0.5 μM (Figure 3a-f). Finally, a third examination involved both entities at concentrations of 1.0 μM (Figure 4a-f). The graphical representations derived from these experiments offer qualitative insights into the interaction dynamics before the quantitative determination of K_D. An increase of each of the substrate loadings shows a clearer emission peak at the YPet fluorescent emission at 475 nm indicating an energy transfer between the two protein pairs.

A select set of E3 ligases were chosen for assessment at lower concentrations of 0.1 μM and 0.5 μM, in addition to the standard 1.0 μM, to discern potential concentration-dependent effects on their interactions. However, all E3 ligases were evaluated at the 1.0 μM concentration alongside the determination of Förster resonance energy transfer (FRET) efficiency (Em_{FRET}) to investigate their binding characteristics comprehensively.

A quantitative absolute FRET signal value, Em_{FRET}, can provide additional information about the binding nature between the E3 ligase and IAV M1 protein. The Em_{FRET} was obtained to determine the sensitized FRET signal resulting from the binding of two proteins. The fluorescent pairs were excited at excitation wavelengths of 414 nm and 475 nm for CyPet and YPet, respectively. The α coefficient, necessary for determining Em_{FRET}, was derived from the donor fluorescent protein excited at 414 nm. Consequently, the β coefficient was derived from the acceptor fluorescent protein excited at 475 nm. The α and β coefficients were multiplied by the fluorescent emission of the donor and acceptor and subtracted by the total emission, as described in Equation 1.

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

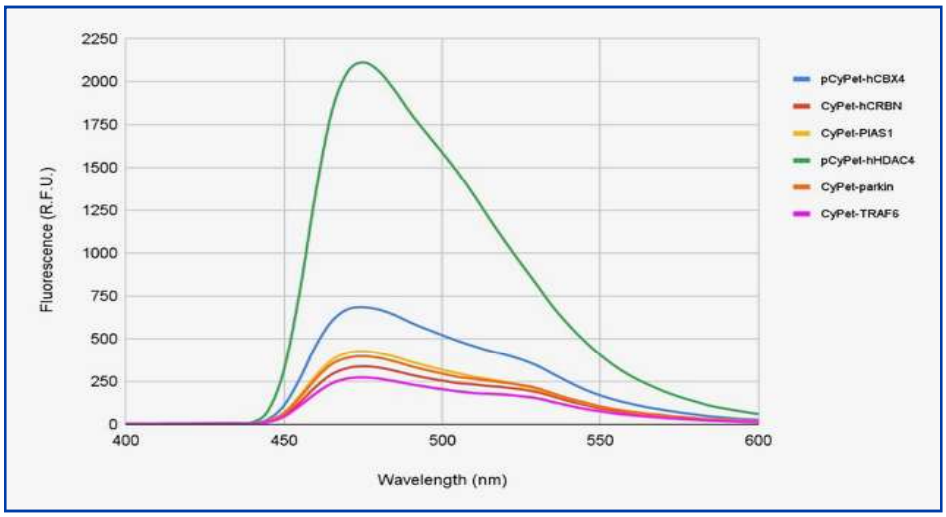


Figure 2: 0.1 uM CyPet : 0.1 uM YPet IAV M1 interaction spectrum graph determined from EmFRET assay. CyPet-hCBX4 (blue), CyPet-hCRBN (red), CyPet-PIAS1 (yellow), CyPet-hHDAC4 (green), CyPet-parkin (orange), CyPet-TRAF6 (pink).

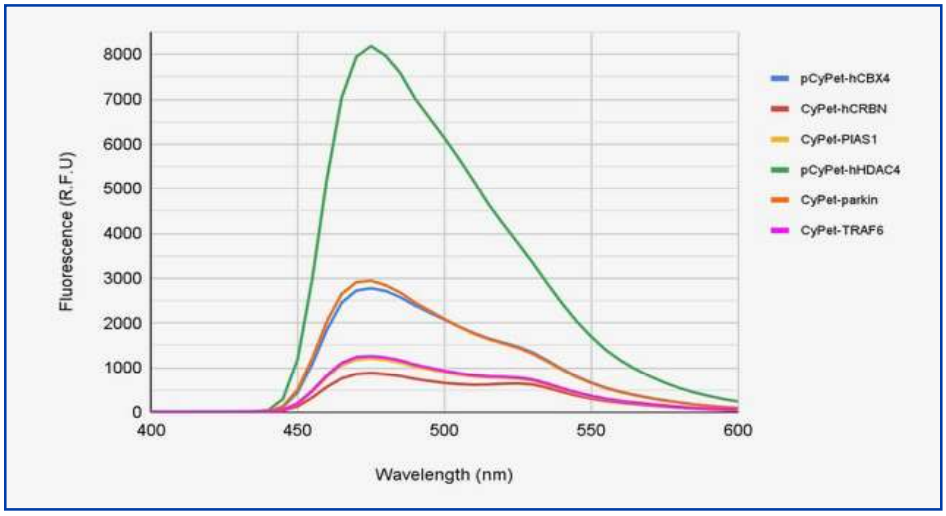


Figure 3: 0.5 uM CyPet : 0.5 uM YPet IAV M1 interaction spectrum graph determined from Em_{FRET} assay. CyPet-hCBX4 (blue), CyPet-hCRBN (red), CyPet-PIAS1 (yellow), CyPet-hHDAC4 (green), CyPet-parkin (orange), CyPet-TRAF6 (pink).

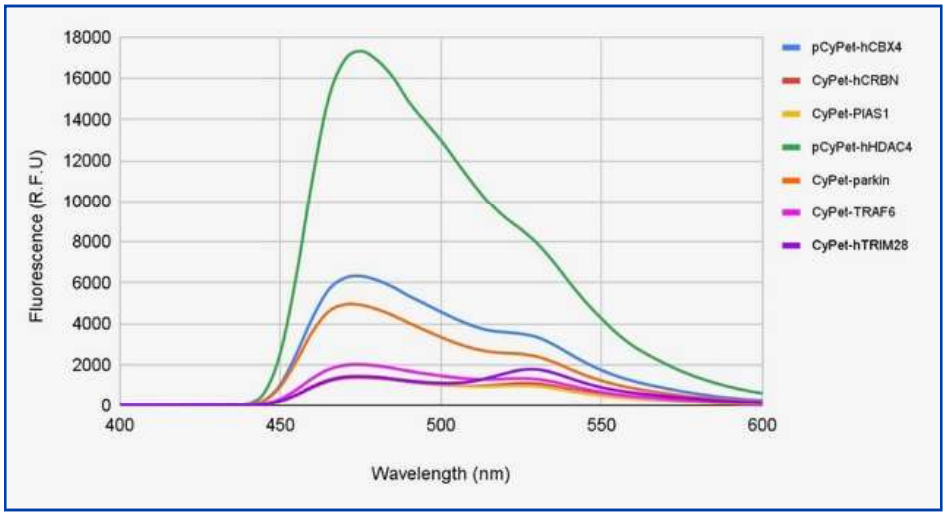


Figure 4: 1.0 uM CyPet : 1.0 uM YPet IAV M1 interaction spectrum graph determined from Em_{FRET} assay. CyPet-hCBX4 (blue), CyPet-hCRBN (red), CyPet-PIAS1 (yellow), CyPet-hHDAC4 (green), CyPet-parkin (orange), CyPet-TRAF6 (pink), CyPet-hTRIM28 (purple).

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

The acquired Em_{FRET} values were then subjected to comparative analysis, facilitating the assessment of the binding affinity between the E3 ligase and the IAV M1 protein. Consistent throughout (Figure 2b, 3b & 4c) there was a clear energy transfer between the pair with an evident peak at 414 nm and 475 nm, however the Em_{FRET} value is considerably low at 89.74 (Figure 5). This comparative evaluation was depicted graphically in Figure 5, offering a visual representation of the binding characteristics between the protein entities under investigation. The Em_{FRET} value for pCyPET-PIAS1 and pCyPet-M1 was 565.92, exhibiting a high binding affinity. In addition, the Em_{FRET} values for PIAS3, PIAS4 hSCNA, and TRM28 were very high too, indicating potential interactions. The generated Em_{FRET} values also provide a clue for further investigations.

We determined the K_D values between PIAS1 and TRIM28, and IAV M1 in order to determine its binding affinity for further characterizations. The binding affinity between the fused CyPet hTRIM28 and PIAS1 with YPet IAV M1 was determined by holding the FRET donor at a set concentration of 0.1 μM. The FRET acceptor was titrated in varying concentrations from 0 to 25 μM. The K_D value was determined by quantifying the absolute FRET signal between the interactions of the two fluorescent pairs.[12] The curves generated in Figure 6 exhibit the binding affinity difference between hTRIM28 with IAV M1 and PIAS1 with IAV M1. The calculated K_D values for (Figure 6a & b) were 24.2 μM and 2.9 μM, respectively. The determined values indicate that the E3 ligase PIAS1 exhibits a notably higher affinity for IAV M1 protein, indicating a real SUMOylation E3 ligase for IAV M1, whereas the E3 ligase hTRIM28 is not. We will examine the E3 ligase activity of PIAS1 in the future study.

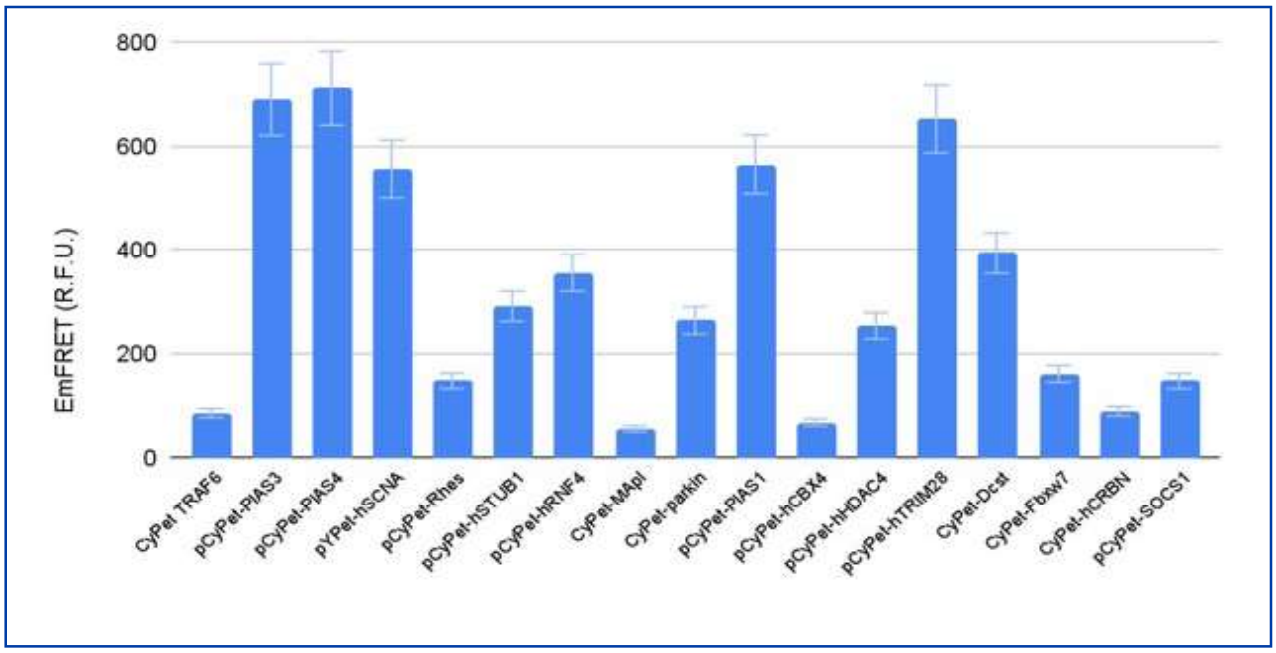


Figure 5: Results of varying 1uM E3 Ligase with 1uM YPet IAV M1 to check for interaction prior to K_D determination.

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

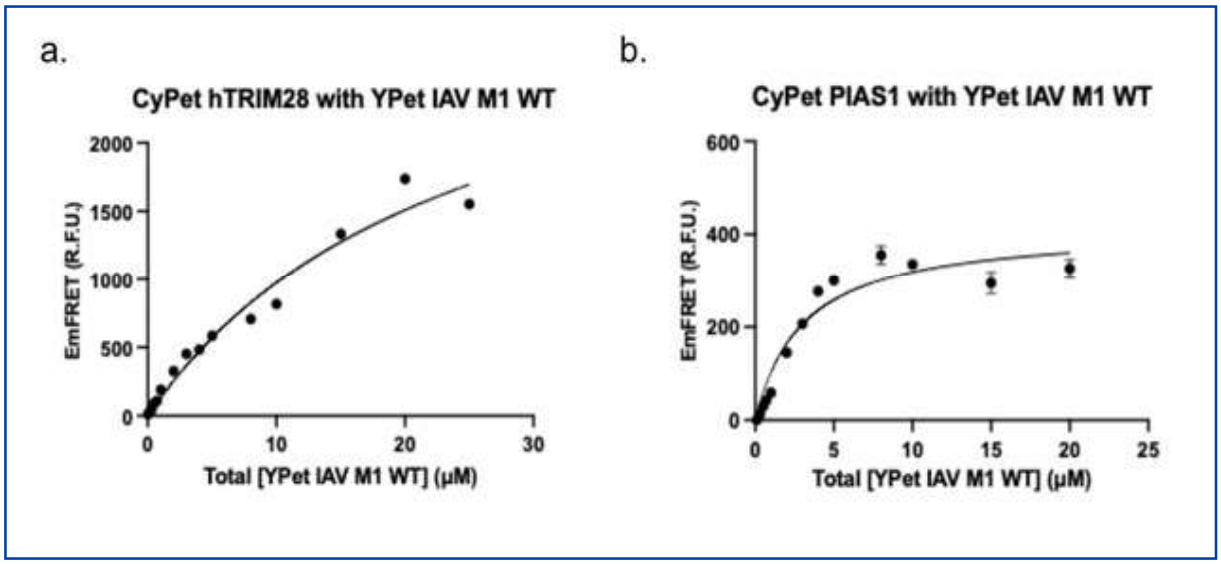


Figure 6: K_D determination results. (A) The interaction between IAV M1 and hTRIM28 was determined, with a K_D value of 24.2 μM. (B) The interaction between IAV M1 and PIAS1 was determined, with a K_D value of 2.9 μM.

DISCUSSION

SUMOylation is a post-translational modification process capable of regulating protein function *in vivo*. Viruses, such as the influenza virus, have been shown to utilize the SUMOylation process to replicate once infecting human cells. Viral proteins exploit the SUMOylation process to enhance their assembly and block the immune response of the host protein.[14] However, similar to the viral protein's ability to utilize the SUMOylation pathway to regulate its replication, the host protein can combat the viral infection by modulating its immune response through SUMOylation. We were able to isolate the fluorescence-labeled proteins of interest for K_D determination to understand their interaction better by taking molecular engineering approaches. After preliminary spectrum and Em_{FRET} analysis, CyPet PIAS1 and hTRIM28 were chosen to determine its K_D because they showed high FRET signals. However, hTRIM28 with the initial high Em_{FRET} value showed a very high K_D value of 24.2 μM, indicating a low binding affinity for IAV M1. Whereas PIAS1 was consistent with its preliminary results and had a low K_D value of 2.9 μM. The results suggest that PIAS1 exhibits a high affinity for IAV M1 protein and could be a real E3 ligase for M1 protein. Once this is validated, chemical inhibitors can be developed to block the host-viral interaction.

We were surprised to find that the same protein appeared to exhibit varying degrees of affinity when comparing protein-protein interactions using three different methods of FRET. Due to the presence of non-specific interactions between proteins, high concentration can force two proteins to interact spatially rather than based on their true interaction affinity. Many types of interfaces exist in physiological fluid media, such as important protein fibers, membranes, and interfaces between condensed phases of solutions, in which interfacial interactions with solute molecules (in this case proteins) in aqueous solution can occur.[5] Depending on the distance between the solute and the surface, the solution layer can be considered close enough to the interface to include a

A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

microenvironment separate from the whole solution. As such, qualitatively assessing FRET interaction alone is not sufficient to accurately determine the affinity between proteins, especially large-sized proteins that are more susceptible to non-specific binding at high concentrations. Unavoidable protein degradation in our expressed protein purification makes the final product contain some non-fluorescent tagged proteins. These proteins also have interferent effects when squeezed in tight spaces, all of which can lead to inaccurate results in the final calculation of FRET values. In measuring the affinity between 1 μM CyPet hTRIM28 and 1 μMYPet IAV M1 WT, an EmFRET of 653 R.F.U. was obtained, especially in measurements using a 384-well plate non-specific binding was observed due to the large size of the molecule and high concentration of CyPet hTRIM28 (145,040 Da). To solve this problem, we used qFRET to measure the affinity of the proteins. The FRET values for true target protein interactions were first calculated by subtracting the interference of nonspecific interactions and non-target protein interactions. Secondly, by gradually increasing the amount of YPet IAV M1 WT and fixing the amount of 0.1 μM CyPet hTRIM28, when the binding between the target proteins reaches saturation, depicted as a plateau, where no more binding can be achieved despite the continuous increase in the amount of YPet IAV M1 WT. Furthermore, at higher concentrations of this protein, it is harder to avoid any non-specific protein-protein interactions that may lead to inaccurate results, as well as saturated fluorescent signals. Therefore, even though strong interaction is observed in measuring FRET alone, more accurate affinity calculations using qFRET ultimately yield substantially weaker interactions among these proteins.

Our study is highly clinically relevant. We aim to elucidate the emphasis on screening for host factors that viruses, such as influenza, exploit for the replication of their genome rather than on the viral protein itself if we are to engineer an avenue of effective therapeutics that bypass viral evasion strategies.[6] While the IAV M1 protein is essential for viral assembly, replication, and spread within the host, its SUMOylation, dependent on host factors such as E3 ligases, may be as crucial for IAV M1 protein function.[7,8] As such, SUMOylation is a multi-step cascade of reactions in which the SUMO post-translational modifier (PTM) is activated and conjugated by a known E1 heterodimer and an E2 enzyme, respectively.[9] Further probing into the importance and mechanism of E3 ligases, which catalyze the transfer of conjugated SUMO to the viral protein (M1), is still lacking, as specific and physiologically relevant candidates have not yet been identified until this screening approach using qFRET. In this study, we clarified the importance of quantitative analysis of these interactions using qFRET, as seen with the aforementioned discrepancy between CyPet hTRIM28 and YPet IAV M1 interaction compared with their biochemical activity. Thus, we have gained insights into yet another potential host factor as a tunable target for developing therapeutics that can modulate and hopefully attenuate influenza replication. However, the degree to which these *in vitro* results agree with *in vivo* interactions remains to be seen; luckily, we present qFRET again as a solution for investigating and comparing the effect of other E3 ligases in both experimental settings.[10,11]

The qFRET technology used in this study is carried out in a solution without a high purity requirement, and this condition can mimic physiological and pathological conditions. Therefore, the qFRET-based measurements are closer to physiological events in living cells. In addition, the qFRET assay is very sensitive, and the concentrations of fluorescence-tagged proteins required in the qFRET assay can be as low as nM; therefore, a minimal amount of proteins is needed for the interaction affinity determinations. Furthermore, the qFRET assay is environmentally friendly and does not contain any radioisotopes or chemicals. Applications of the qFRET technology should provide high-quality

protein interaction and catalytic affinities of systems, networks, and proteomes and provide comprehensive quantitative biological and biomedical maps without the need for laborious protein purification, especially for those difficult-to-be-expressed proteins, such as SUMOylation E3 ligase in this study. The genome-wide search for SUMOylation E3 ligase for influenza virus M1 protein is not only important for research but also for novel anti-virus therapeutics development in the future.

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A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

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Pacing - Slowing Phenomenon in Varying Length Tasks

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Elliot Randolph, Department of Biology
Hunter Sturgill, Ph.D. Candidate, Department of Psychology
David. A Rosenbaum, Ph.D., Department of Psychology

ABSTRACT

Pacing is a critical mechanism for regulating effort over time to achieve optimal performance. While commonly associated with sports, pacing can be seen in individuals' everyday lives. For example, students can pace their studying habits to retain information effectively and avoid last-minute studying. Current pacing literature lacks empirical testing in less physically demanding tasks. The team sought to study pacing, using a task analogous to running different-length events. In two preliminary experiments, undergraduate students pressed the enter key N = [8, 16, 32, 64] times. Running and key presses involve prolonged repetitive activities that require sustained performance. Participants were instructed to complete the task rapidly and exceed the specified number of taps, similar to runners sprinting through the finish line. Our main method of measurement was the mean interresponse interval (IRI) which is the average elapsed time between consecutive taps. Our main question was if participants would tap slower when N is large versus when N is small, just like runners. Surprisingly, participants did not change their performance based on N and slowed dramatically as they approached N. The premature slowing effect was indicated by an appreciable difference between IRI values for Experiment 2 where $F(3,656) = 3.83$, $p = .0097$. We varied the count feedback between experiments: either counting up from 1 or down from the total number of key presses required. The slowing effect before the trial ended was not extinguished. This premature slowing suggests that as people approach the end of a task, they slow their performance.

KEYWORDS: pacing, meta-cognition, learning process, slowing effect

FACULTY MENTOR - Dr. David. A Rosenbaum, Department of Psychology

David A. Rosenbaum, Ph.D. is a Distinguished Professor in the Psychology Department (in the Cognition and Cognitive Neuroscience area). He recently was honored by being invited to publish Cognitive Control of Action: Selected Works of David A. Rosenbaum in the World Library of Psychologists Series (Routledge Psychology Press, 2024), being one of only 24 psychologists in all areas of psychology to publish a collected-works volume since the series began in 2013.

UDITI DESAI

Uditi Desai is a fourth-year Neuroscience major studying pacing in everyday tasks under the supervision of Dr. David A. Rosenbaum and mentor, Hunter B. Sturgill. Additionally, Uditi is conducting research on QTPoC populations and mental wellness, under the supervision of Dr. Nicholas Napolio. The QTPoC Project has funding from UCR's Basic Needs Department and LGBT Resource Center. She is a part of several student organizations and is working towards a career as a physician.

ELLIOT RANDOLPH

Elliot Randolph is a fourth year Honors Biology major. For the past two years, he has worked as a research assistant in Dr. David Rosenbaum's Laboratory for Cognition and Action, under the mentorship of Ph.D. Candidate Hunter Sturgill. Elliot also works in hospital settings as a Cardiology Research Fellow and as an Internal Medicine Volunteer from the Internal Medicine Department at UCR. In the future, Elliot aims to pursue a career as a surgeon.

Pacing - Slowing Phenomenon in Varying Length Tasks

INTRODUCTION

Homeostasis, the body's equilibrium of internal conditions, is essential for health and well-being (Billman, 2020). Maintaining this equilibrium is done through various methods, one of which is the pacing of one's performance. Pacing in daily activities allows the body to sustain its internal balance throughout the day and prevent excessive depletion of resources. Thus, as individuals engage in tasks throughout the day, they adjust their efforts to optimize performance while conserving energy (Menting, 2022).

Folklore and Pacing

Pacing has been seen in folk knowledge and many well-known idioms, emphasizing its prevalence, such as "haste makes waste," "take it a day at a time," and "fools rush in." The most famous idiom, "slow and steady wins the race," comes from Aesop's famous fable "The Tortoise and The Hare." The tortoise, pacing itself throughout the race, won despite the hare being a speedy animal.

The concept of pacing is not limited to specific timeframes—it involves spreading performance out over time and condensing it into shorter bursts within time intervals (Gersick, 1994). For instance, when studying for an exam, pacing might mean spreading out study sessions over several weeks to avoid cramming. Pacing could also entail focusing intensely for shorter periods, such as using the Pomodoro Technique, where an individual may work for 25 minutes and then take a 5-minute break. By alternating between periods of focused work and brief breaks, the Pomodoro Technique helps individuals pace themselves effectively, manage their energy, and maintain productivity throughout the day (Cirillo, 2013).

A study focusing on the impact of massed versus spaced learning revealed that spaced studying resulted in better test performance compared to massed studying (Kornell & Bjork, 2008). Thus, pacing oneself during learning tasks might also lead to better understanding and retention of information.

Similarly, pacing strategies can be manifested in the athletic world. Spreading out study sessions over several weeks is similar to how marathon runners approach pacing. But working intensely in short bursts, such as by using the Pomodoro Technique, is similar to how sprinters approach their races.

Pacing studies predominantly focus on athletics, where it guides athletes' strategies and performances. From sprinters to marathoners, a mastery over pacing is essential for optimizing energy expenditure and achieving peak results (Abbiss, 2008). There is a distinction between pacing as a rate and pacing through decisions. For example, compare sprinting to long-distance running: in sprinting, the focus is on maintaining a high pace for a short duration, while in long-distance running, it is about sustaining a consistent pace over a longer period by making strategic decisions about energy expenditure and effort allocation. In a 1992 study, van Ingen Schenau et al. noticed that sprinters running the 1000m versus the 4000m adopted different pacing strategies.

Our project delves into the pacing behavior in every day, less physically demanding tasks. We aim to understand whether similar pacing strategies manifest in daily task contexts compared to pacing seen in the athletic world. Specifically, we want to investigate whether participants modulate their performance as the length of a task changes. By exploring pacing behavior in everyday tasks, we aim to contribute to a deeper understanding of human behavior, productivity, and well-being.

Pacing - Slowing Phenomenon in Varying Length Tasks

Relevant Research and Implications

A relevant study hypothesis that may aid in our understanding of pacing is the goal gradient hypothesis. The goal gradient effect refers to the phenomenon where an increase in efforts is seen as the goal is approached. This study was done on rats and noticed that as the rats get closer to reaching a goal, they tend to work harder and more efficiently (Hull, 1932). In addition, pacing patterns in activities like running, swimming, and cycling are influenced by a psychological mechanism called "perceived impact." This means that athletes push harder toward the end of a task when they feel their efforts have a bigger impact on their advancement (Emmanuel, 2019). Perceived impact enhances the goal gradient hypothesis by elucidating how individuals increase their effort as they approach a goal, driven by the belief that their actions have a greater impact on their progress.

The goal gradient hypothesis suggests that as individuals get closer to a reward, they expend more effort to reach it. Other studies have built on this idea, proposing new insights into how humans respond to rewards. Through various experiments and analyses, they found that participants in a café reward program increased their coffee purchases as they neared a free coffee reward (Kivetz et al, 2006).

The "labor-in-vain" effect suggests that individuals may pace themselves differently depending on the difficulty or intensity of a task (Nelson, 1988). For example, they might slow down or take breaks during more challenging activities to conserve energy. This suggests a modulation of activity depending on the task length and difficulty.

The goal gradient hypothesis suggests that as participants near the end of a trial, they may ramp up their efforts, echoing findings from studies by Kivetz et al. (2006) and Emmanuel (2019). Various potential explanations for this hypothesis exist in varying fields of psychology. One of these explanations revolves around prospect theory, which employs the concept of diminishing sensitivity to argue that the value of each action increases as the goal draws nearer (Heath et al., 1999). According to Koo and Fishbach (2012), goal outcomes hold greater value when they are in proximity to the goal's end state, as the value function becomes steeper near this point. Gestalt psychology also contributes to understanding this phenomenon, suggesting that motivation increases as individuals strive to achieve closure (Zeigarnik, 1938). Lastly, another perspective in social psychology posits that each step towards goal attainment is perceived as a success, with the value of each success increasing as it contributes further to reaching the goal (Förster, 1998).

Additionally, insights from Nelson (1988) regarding the labor-in-vain effect indicate that participants may adjust their pacing based on the length of the trial. Considering the implications of the Pomodoro Technique by Cirillo (2013) and the results from massed and spaced learning by Kornell & Bjork (2008), we observe that pacing strategies can effectively influence participants' performance across various tasks and learning contexts.

Research Question

Unlike most pacing studies, this research doesn't involve physical exhaustion. Instead, it focuses on the modulation of performance in a sustained task. This study aims to investigate whether pacing strategies observed in physically demanding tasks, such as running or cycling, are similar in a less physically demanding task, specifically keypressing. We vary the duration of the task to attempt to answer the question: does the pacing mechanism seen in physically demanding tasks work similarly in non-physically demanding tasks? If so, then similar variations in performance given the distance of the event, so to speak, should result. We sought to test this with the simplest task

we could devise, a button-pressing task. Both running and keypressing share similarities as they both involve constant repetitive activity over a duration of time.

METHODOLOGY: EXPERIMENT 1

Participants

A total of 45 undergraduate students participated in this research study from the University of California, Riverside (UCR). All participants were obtained from the undergraduate research pool using the Sona platform for psychology courses PSYC001 and PSYC002.

Procedure

We sought to study pacing using the simplest task we could. Participants in our study were instructed to press the enter key of a standard computer keyboard a specified number of times in each trial (N = 8, 16, 32, 64). There were a total of 32 trials with each N value being seen a total of 8 times each. For each event or value of N, participants were directed to continue tapping past the trial length, akin to a runner sprinting through the finish line. Additionally, participants were told there would be no penalty for tapping beyond the N required taps. Real-time countdown feedback regarding the number of taps was provided until each participant reached zero responses left, eliminating the need for them to keep count of their responses. Feedback, including the average response time per trial and the best overall time of any trial was given to encourage participants to go as quickly as possible. MATLAB software administered the experiment and recorded the data. Inter-response intervals (IRIs), measured as the elapsed time between successive taps, were the variable of interest and represented the speed of the performance.

Hypotheses

The primary question of this study was whether participants would modify their performance as the length of the task changed. We hypothesized that if pacing is a cognitive mechanism that works irrespective of a task's physical demands, then participants' IRIs should change as a function of N (the required number of responses). Alternative predictions were guided by the previous literature mentioned in the introductions and we propose those predictions below.

Based on the goal gradient hypothesis, we posit that participants will tap faster as they approach the end of each trial due to an end-of-trial speed-up phenomenon. It is also plausible that participants will exhibit a gradual increase in speed as the length of each trial progresses, as referred to by the perceived impact mechanism. The final prediction is based on the scenario of the "labor-in-vain" effect where participants will exhibit a consistent tapping pace but may slow down, particularly in trials that require lots of taps and not in shorter events.

Measures

In this study, we employed several measures to assess participants' performance in response to varying trial lengths but primarily cover one in this article. Firstly, the total number of responses (N) served as our key independent variable, with trial lengths ranging from 8, 16, 32, and 64. Concurrently, the number of between-response intervals (n) was recorded and represented the speed at which participants performed. Smaller response intervals mean quicker taps and, if sustained, shorter overall times. The study recorded the mean inter-response intervals (IRIs) which is the average elapsed time between consecutive taps for that event. Events were pseudo-randomly ordered, ensuring that no event was tested twice before completing all four N values.

Pacing - Slowing Phenomenon in Varying Length Tasks

RESULTS: EXPERIMENT 1

The main question for this study was whether or not participants would modify their performance when the length of a trial varied. If the mechanism of pacing is the same regardless of the physical demands of the task, then we expect to see different average speeds depending on the length of the events. In Figure 1 we show the average between tap time, or speed, for each of our 4 events. Noticeably there is little difference between each of the bars. The lack of difference between events was confirmed using a one-way analysis of variance (ANOVA) for each of the 4 events, $F(3,176) = 0.04$, $p = 0.988$.

This omnibus data helps to answer our question regarding changes in performance by event length but does not speak to our alternative predictions. Showing the data another way, in Figure 2 the mean inter-response time for each of the intervals of an event was averaged across participants and blocks. We see that

We see that participants did not perform differently in each of the 4 events, with no appreciable difference between the lines of different colors. However, we do see a change in the performance across the event. IRIs quickly shrink after the first response or two and then gradually increase as more responses are required. This was confirmed by an ANOVA for the first 4 IRIs by the 4 events where the interaction between event and response number could have occurred by chance, $F(3,704) = 0.3$, $p = .83$. Additionally, the main effect of the event was not appreciable, $F(9,704) = 0.1$, $p = 0.99$, which also confirms the previous figure's result. However, there was an appreciable difference between the average time for the first 4 intervals, $F(3,704) = 3.45$, $p = 0.016$. A multiple comparison test revealed that the difference was between the first IRI and both

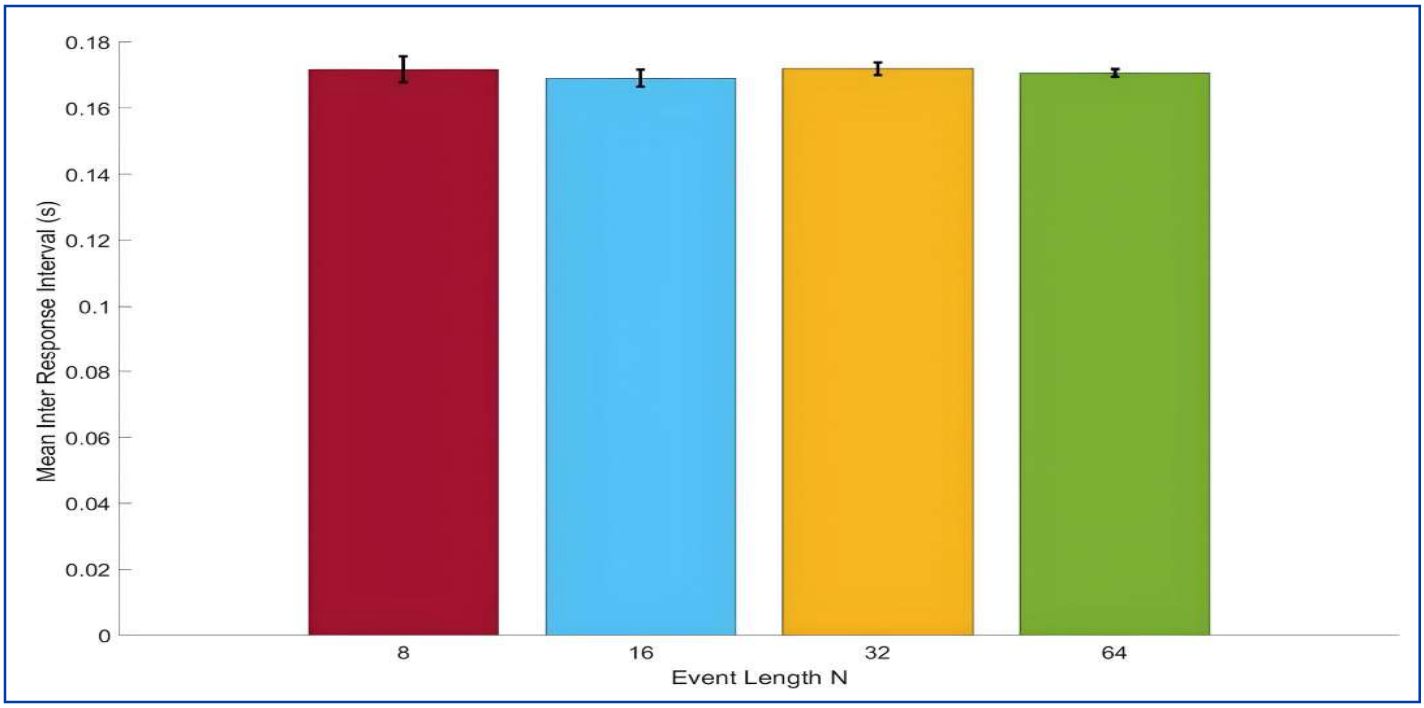

Figure 1: Mean Inter-Response Intervals by Event

Note. Mean Inter-Response Interval (± 1 SEM) in seconds for each of the 4 events averaged over the 8 blocks, all intervals (n), and overall 45 participants. The number of required taps distinguishes each event and is not only shown in different colors in this and all figures following but is also denoted by the value on the x-axis.

Pacing - Slowing Phenomenon in Varying Length Tasks

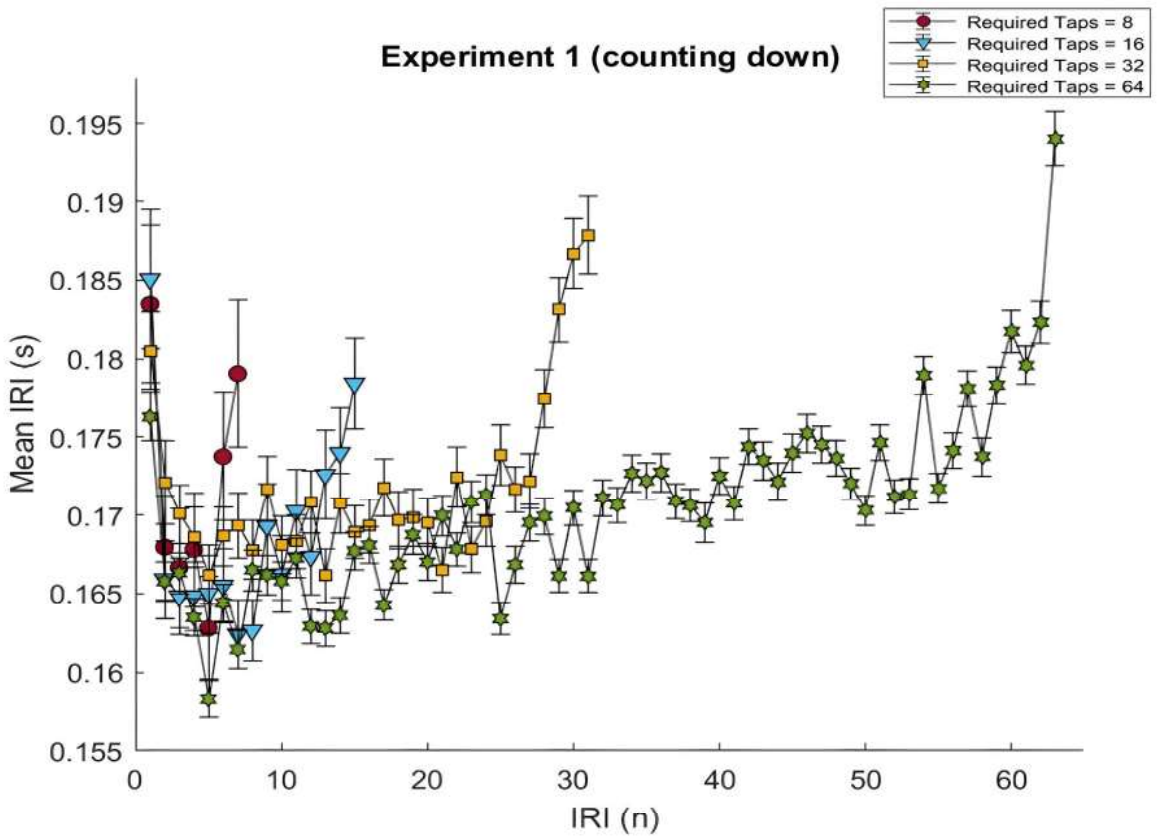

Figure 2: Mean Inter-Response Intervals

Note. Mean (± 1 SEM) inter-response intervals (IRI) as a function of which interval (n) for each of the 4 number of required taps or events. Both the marker color and the marker shape represent the event length.

the third and fourth intervals. This shows that there is an appreciable increase in speed during the first few taps of an event. Lastly, and most surprising was the sharp decrease in speed (increase in response intervals) toward the end of the trial. This feature of the data was not hypothesized or expected and so we respectfully chose not to perform hypothesis tests like those covered above.

DISCUSSION: EXPERIMENT 1

In this experiment, we looked at the effect of trial length on participant performance when asked to tap a computer key as quickly as possible. Our question was whether participants would change their tapping rate when the number of required taps changed. We hypothesized that if pacing one's performance is done irrespective of the physical demands of the task then key pressing should work similarly to running. Our data showed that participants did not modify their performance as a function of N. However, they did show increases in speed and dramatic slowing as the trial began and ended, respectively. We did not hypothesize the dramatic slowing that participants showed at the end of each event, thus no significant data for it was collected in Experiment 1. However, this slowing effect goes directly against the goal gradient effect popularized by Hull (1932) and others. We believe

this end-of-trial slowing could be an artifact of the feedback provided to the participants. In each trial, participants saw the number of required taps count down from N to 0 as they submitted more and more key presses. We speculated that this counting down to 0 inhibited participants from sprinting through the finish line as instructed thus not maintaining their previously set level of performance. We sought to test this theory in the experiment to be covered next.

METHODOLOGY: EXPERIMENT 2

Participants

For Experiment 2, 42 undergraduate students participated for partial course credit. We recruited participants using the same recruiting system, Sona.

Procedure

In Experiment 2, we aimed to address the observed end-of-trial slowing by altering the count feedback provided to participants. Instead of having a countdown method, participants were shown feedback that counted up during each trial, beginning from "1" and incrementing with each tap. Participants were given the same instructions as Experiment 1 of tapping the enter key as fast as they could for a predetermined number of times in each trial, with N values set at 8, 16, 32, and 64. Each N value was tested 8 times across 32 trials, and the experiment's events were pseudo-randomly ordered. Real-time feedback on the number of taps was provided to participants, although differently than before. End-of-trial feedback with the current time and best overall time was also provided. All aspects of the study were the same except for the performance feedback regarding the number of taps submitted (n) out of how many were due (N). The study again recorded the inter-response intervals (IRIs), which was the elapsed time between two consecutive taps.

Hypotheses

If pacing is a cognitive mechanism that works independently of the physical demands of a task, then participants' IRIs should change as a function of N, the required number of responses. The slowing seen in Experiment 1 may or may not have been due to feedback given to participants, thus we presume changing the feedback provided might eliminate the end-of-trial slowing. Our hypotheses align with those proposed in Experiment 1, except for the aforementioned end-of-trial slowing. In Experiment 2 we are replicating the test regarding event length and performance changes and extending to test whether the end of trial slowing was due to an artifact of the design. interval which was averaged to varying degrees.

Measures

Quantitative measures remain the same from Experiment 1 where (N) represents the number of total responses required for the trial our independent variable, (n) represents one of N-1 inter-response intervals. The dependent variable is the inter-response interval which was averaged to varying degrees.

RESULTS

Figure 3 shows an omnibus graph model depicting the event length (N) on the x-axis and the mean inter-response interval in seconds on the y-axis. Based on the omnibus comparison across different trial lengths, there is no significant variation observed in the Mean Inter-Response Interval (IRI) as trial lengths increase. A one-way ANOVA showed no appreciable difference between the four events for Experiment 2, $F(3,164) = 0.27$, $p = 0.85$. We again chose to look at the mean interval data for each (n) in Figure 4, displaying the mean IRI changes across different trial lengths.

Figure 4 also shows that participants performed similarly, with no appreciable difference, in each of the 4 events. A 4x4 analysis of variance uncovered a

Pacing - Slowing Phenomenon in Varying Length Tasks

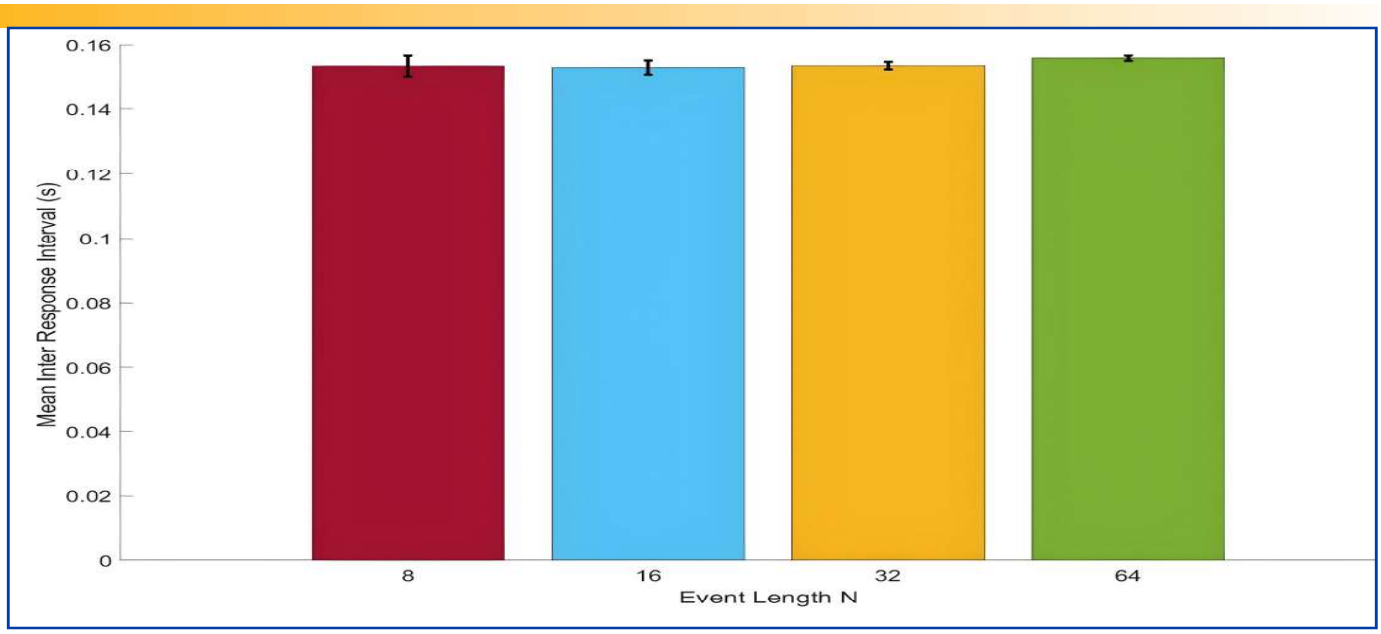

Figure 3: Mean Inter-Response Intervals by Event

Note. Mean Inter-Response Interval (± 1 SEM) in seconds for each of the 4 events averaged over the 8 blocks, all intervals (n), and overall 42 participants. The number of required taps distinguishes each event and is not only shown in different colors in this and all figures following but is also denoted by the value on the x-axis.

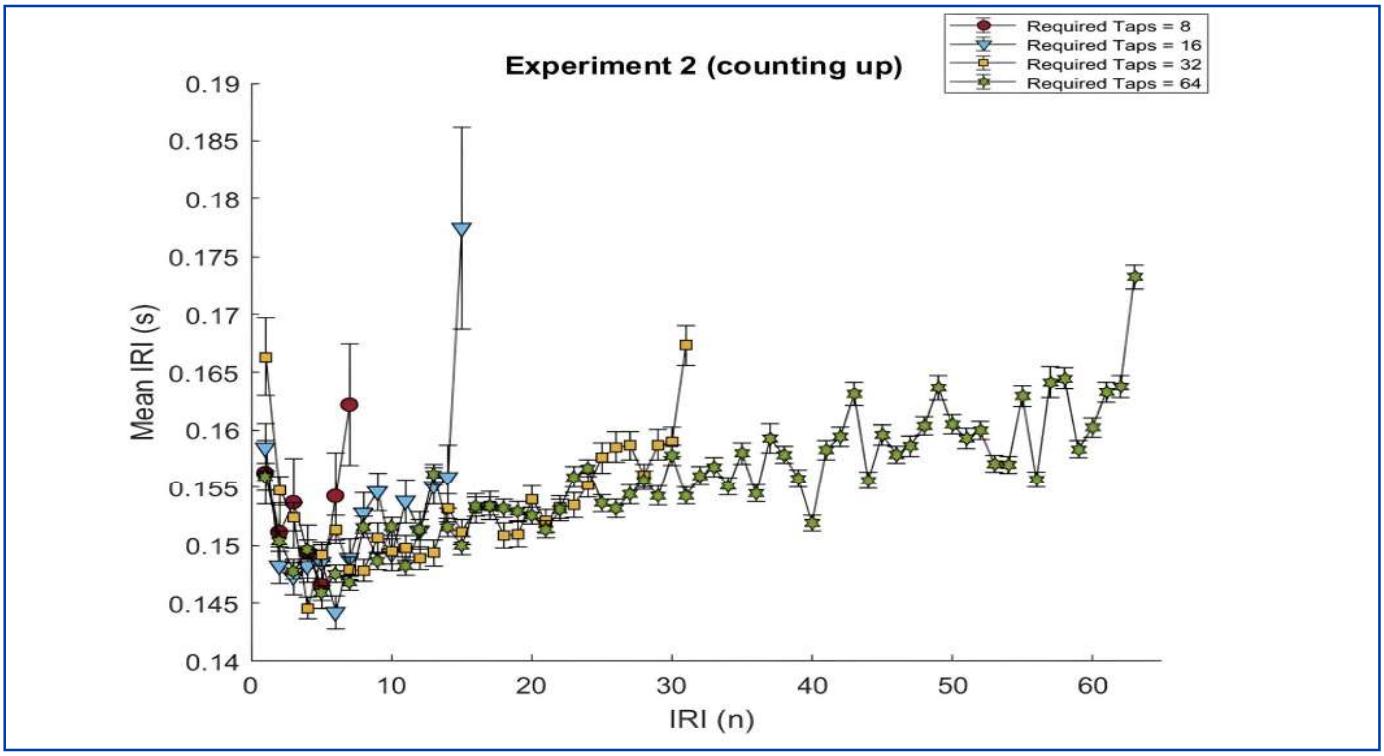

Figure 4: Mean IRI Values for Actual Responses (n) of Each Event in Experiment 2

Note. Mean (± 1 SEM) inter-response intervals (IRI) as a function of which interval (n) for each of the 4 number of required taps or events. Both the marker color and the marker shape represent the event length.

significant distinction for the first 4 IRI(n) of each event, ($F(3,656) = 6.66$, $p < .001$), replicating the result from Experiment 2. Additionally, there was no notable difference for the event ($F(3,656) = 0.89$, $p = .445$), or the interaction between the event and IRI(n) ($F(9,656) = 0.77$, $p = .645$) just as there was in Experiment 1. Figure 4 displays that, on average, each trial rose by ≥ 0.02 seconds, comparing the start point and the end point of each N value. At the beginning of each event, the IRI rapidly decreases after the first few taps; however, the IRI then gradually increases, and at the very last response, the IRI has a sharp increase. An increase in IRI shows a slow-down in performance and confirms that this effect was not related to the feedback change from counting down to 0 to counting up to N. This was confirmed with an ANOVA test for the last 4 IRIs. The analysis showed that the main effect for Event, $F(3,656) = 3.83$, $p = .0097$ and IRI(n), $F(3,656) = 8.0$, $p < 0.001$, indicating statistical significance. However, the Event and IRI interaction was not significant ($F(9,656) = 0.5$, $p = .875$).

DISCUSSION: EXPERIMENT 2

The results from Figures 3 and 4 show that there was an increase in IRIs as the trial length increased. This could imply a slower pace or a longer pause between taps, as the trial length increased. Our expectation based on previous literature was that participants would increase their pace–lower IRIs–as they approached the end of the trial. Instead, participants slowed down and had much larger IRIs as they approached N. This experiment aligns with the end-of-trial slowing in Experiment 1. Both experiments showed this remarkable phenomenon that is in direct conflict with the goal gradient prediction. In both Experiments 1 and 2, participants may have experienced cognitive and/or motor fatigue as they progressed through longer trial lengths, leading to a natural slowdown in tapping speed as trial length increased but they also showed a drastic slowing increase in the last few taps of an event.

GENERAL DISCUSSION

The Pacing Project aims to explore pacing in everyday tasks. Previous studies done in the athletic world show that individuals have different pacing strategies for different events such as sprints and marathon running. Our primary objective was to see if individuals would modulate their performance as the length of an event changes. Thus, we designed an experiment where participants were to tap the enter key a various number of times, ($N = 8, 16, 32, 64$). We hypothesized that participants would have different pacing strategies for the different trial lengths, and that they would manifest in the intervals of the tapping. In both experiments covered here, participants tapped the enter key as quickly as possible and were asked to maintain that tapping even beyond the required amount, just as runners run through the finish line of a race. The results were replicated in both experiments where participants did not appreciably modify their performance for each event. However, they did speed up and slow down appreciably at the beginning and end of each event. Most surprisingly, as participants approached the end of a trial their tapping slowed drastically.

This discrepancy between instruction and behavior creates a form of cognitive dissonance, where individuals may experience discomfort or conflict due to the inconsistency between what they are told to do and what they actually do. This observation raises questions about the underlying factors influencing participants' pacing behavior and suggests potential complexities in how pacing is executed and understood in this context.

The end-of-trial slowing was directly tested between the two experiments by modifying the during-trial feedback that showed participants how they were performing by visually counting out their taps on the screen. This discovery is in direct conflict with the long-standing

goal gradient effect from Hull (1932) and other experiments that support the goal-gradient hypothesis as seen from Emmaneul (2019) and Kivetz et al. (2006). Additionally, our results having no appreciable difference between trial lengths go against the insights from Nelson (1988) regarding the labor-in-vain effect. This end-of-trial slowing will be our main focus to address in future experiments.

FUTURE DIRECTIONS

Following discussions with research assistants and participants, a new hypothesis emerged regarding the feedback provided after each trial regarding participants' performance. It was suggested that participants might experience apprehension about surpassing the required number of taps, fearing they could miss out on feedback. While this concern wasn't applicable due to the program's design, participants' uncertainty about what to expect prompted us to take their feedback into account. Thus, we aimed to address this aspect in subsequent experiments. We believe that encouraging participants to tap at their comfortable pace, rather than as fast as they can, may alleviate this apprehension. By allowing participants to define their own comfortable pace, we aim to observe whether they will naturally increase their speed towards the end of the trial, rather than experiencing a slowdown. This adjustment is intended to eliminate the observed slowing-down effect and provide insights into participants' pacing behavior.

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Pacing - Slowing Phenomenon in Varying Length Tasks

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Exploring the World by Touch: Guidance in Bimanual Haptic Search

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ABSTRACT

While visual attention has been extensively studied, haptic attention has remained relatively unexplored. Haptic attention is an integral facet of everyday life, often arising in everyday activities like feeling for a pencil in a backpack or searching for keys in one's pockets. We sought to understand how proprioception—our body's position in a three-dimensional space—and the features of an object (such as variation in length or diameter) contribute to the efficiency of bimanual (the use of two hands) haptic search in an unrestrained environment. We hypothesized that the physical properties of an object, along with the areas in which we search for something—our frame of reference—affect search efficiency, which we quantified via search times. Our study required participants to search for a target item among a set of distractor items without the use of vision, either in a single container with hands coupled or in separate containers with hands separated. We found that bimanual search in one container was not reliably different from bimanual search in two containers. We also found that there was an additive effect of diameter and length discrimination on search efficiency. This effect pertained to length searches always taking longer than diameter searches within the conditions.

KEYWORDS: haptic search, bimanual search, frames of reference, proprioception, attention, features

FACULTY MENTOR - Dr. David. A Rosenbaum, Department of Psychology

David A. Rosenbaum, Ph.D. is a Distinguished Professor in the Psychology Department (in the Cognition and Cognitive Neuroscience area). He recently was honored by being invited to publish Cognitive Control of Action: Selected Works of David A. Rosenbaum in the World Library of Psychologists Series (Routledge Psychology Press, 2024), being one of only 24 psychologists in all areas of psychology to publish a collected-works volume since the series began in 2013.

ALINA AHMED

Alina is a fourth-year student, pursuing a degree in Psychology. She has worked as a research assistant in Dr. Rosenbaum's Laboratory for Cognition and Action for one year under the guidance of Ph.D. Candidate Hunter Sturgill. Alina is currently the President of the Pre-Physician Assistant organization and the Secretary for the Pakistani Student Association on campus. She is also employed as a medical scribe at the Children's Hospital of Orange County. She plans on pursuing a career as a Physician Assistant (PA) and anticipates being a part of the next generation of medical providers.

ADAM BABESHKOV

Adam Babeshkov is a third-year Neuroscience major. He has worked as a Research Assistant in Dr. Rosenbaum's Laboratory for Cognition and Action for two years under the guidance of Ph.D. candidate Hunter Sturgill. Adam aims to pursue a Ph.D. in Neuroscience and build a career in research after graduating.

Exploring the World by Touch: Guidance in Bimanual Haptic Search

INTRODUCTION

Imagine texting a friend while reaching into your pocket to grab your car keys. Or, imagine driving while trying to grab your backpack in the back seat. In both cases, you search with your hand without the benefit of vision. Vision is a complex, important, and advanced human sense that has been extensively studied due to it being a primary driver in our daily lives (Gerrig & Zimbardo, 2008). Conversely, touch, and more specifically unconstrained haptic search, has been less studied (Rosenbaum, 2017).

There is precedence for research on haptic search, however. In a study that is particularly relevant to our present research, Overvliet, Smeets, and Brenner (2018) created a bimanual search experiment exploring the differences of finger use in three conditions: one finger, multiple fingers of one hand, and free use of both hands. They aimed to find out how quickly a target could be found while vision was obscured via an eye mask. Participants were asked to identify a target item—a cylinder, bar, or a rotated cube—placed within a set of distractor cubes that were fixed in a grid. The authors found that search was quickest for the cylinder, as it was the item most different from the distracting cubes. Additionally, Overvliet et al. (2018) found that searching with both hands was quicker than searching with a single hand in all target conditions and that one-finger search was slowest of all in all conditions. The authors concluded that searching with separated hands afforded a division of labor, where each hand could divide the objects amongst them and access each object simultaneously.

Overvliet et al. (2018) creates a great foundation for understanding haptic search, but we wondered whether the main result would remain in a more natural setting. In real life, objects to be felt do not sit affixed to boards in nicely gridded patterns. Instead, they may be piled up or scattered to occupy separate areas. We sought to address this idea with the creation of a more naturalistic task in our experiment. In addition to the idea of naturalism of the earlier work and its reliance on a grid, another feature of the study by Overvliet et al. (2018) interested us. It was unclear whether the spaces in which the two hands did their searching were functionally shared or separate. Other foundational literature speaks to this issue. Squeri and colleagues (2010) designed a bimanual haptic experiment using coupled and uncoupled hands as their conditions. The authors showed, by relying on Bayesian analysis—a statistical approach integrating prior knowledge with observed data, enabling researchers to address uncertainties and present probabilistic inferences—that a shared frame of reference aided haptic search. In their experiment, which also obscured vision, participants indicated which of two pathways felt more curved via touch. Higher levels of accuracy could be achieved when the hands were coupled—or when the two pathways to be felt occupied a space that could be explored by both hands. In this case, a shared frame of reference could improve haptic perception of curvature.

In retrospect, the two studies reviewed above can be said to have had opposing results, as one study supports the greater efficiency of separated hands in search tasks while the other favors coupled hands. The first study may suggest that haptic identification benefitted from separated hands due to the division of labor while the second study may suggest that haptic identification was enhanced by coupled hands due to the opportunity for redundancy. Here we seek to test just that, using a naturalistic haptic search task which we referred to as "free-range haptic search."

In addition to testing frames of reference and perceptual redundancy, we sought also to test the physical features of an object and their influence on search times. Our inspiration came from classic work on visual search by Treisman and Gormican (1986). Through a series of experiments, the authors recorded

the time it took for participants to indicate whether visual targets were present among visual distractors. The authors found that larger targets among smaller distractors were found quicker than smaller targets among larger distractors.

Our study continues the research completed by Sturgill and Rosenbaum (in review) which pursued an analogous outcome in touch, but within a more natural haptic-search environment. They had participants hunt for a 1-inch long plastic pipe among a variable number of distractor pipes, all of which were shorter or longer than the target by the same length difference. The diameters of the pipes were the same in all conditions. The primary discovery revealed that the search time for a consistent 1-inch target varied based on its size relative to other objects. This relationship between target and distractor items, referred to as the relative size ratio from here on,was quantified using the formula: (*max*_length - *min*_length)/*min*_length). The data was effectively represented by a power function, illustrating the dependency of search times on the relative size ratios between targets and distractors. Notice that, for this function, as min_length increases, the ratio gets smaller, so as in Treisman and Gormican (1986), it took less time to find the target when it was larger than the distractor (when the target had max_length). This reduction of the search time was larger with the greater the difference between max_length and min_length.

HYPOTHESES AND PREDICTIONS

In the present study, we extended the earlier work of Sturgill and Rosenbaum (in review) in two ways. One was to add another dimension to the search difference—including pipe diameter to the existing length searches. The other was to use two-hand search in two different areas (separate frames of reference) or together in one area (shared frames of reference).

Our first hypothesis concerned bimanual haptic search and, more specifically, whether searching in one space with two hands, or searching in two spaces with two hands, differed in terms of search efficiency. If haptic search benefits from a shared frame of reference and from redundant tactile sampling of any given object, then search should be most efficient in the single search space in accordance with Squeri et al. (2012); in our experiment, search area was the Tupperware® container. On the other hand, if haptic search benefits from division of labor, then distinct search spaces should be most efficient as seen with Smeets & Brenner (2008).

Our second hypothesis concerned target features and their relation to distractors. We sought to test this relation by controlling for the relative size ratio from previous research which best predicted search times. In addition, we varied the feature—length or diameter—that distinguished the target. If search is guided by the relative difference between the target and distractor, then there should be no difference in search efficiency between our conditions as the ratio was held constant. However, if search is guided differently depending on the feature, then the data should show varying search times depending on the condition.

METHOD

Materials and Apparatus

For the experimental setup, all search items were found inside the plastic Tupperware® containers. Depending on the condition, participants either conducted searches with their hands divided between two containers, each measuring 6x6 inches, or they did a combined search in one container, measuring 12x6 inches. As it was important to ensure participants could not see the search materials, a black poster board was placed above the search area.

Exploring the World by Touch: Guidance in Bimanual Haptic Search

In the container(s), search items consisted of PEX pipes, which are primarily used for plumbing purposes and easily purchased at any local hardware store. In every condition, there was 1 target item and 5 distractor items which varied from the target in either length or diameter—the target item was either shorter/longer than the distractor items or the target item was either wider/thinner than the distractor items, never both (see Figure 1, right panel). Lengths included either 0.50 inches, 0.75 inches, 1 inch, or 1.50 inches. Diameters included either 0.50 inches or 0.75 inches. To ensure that the relative size ratio was controlled and kept constant in the experiment, the 0.5-inch and 0.75-inch items were always tested together, and the 1-inch and 1.5-inch items were always tested together.

Before commencing and immediately after concluding each search task, participants utilized a 3" x 4" metal touchpad to record individual trial search times, shown in Figure 2 (top panel). The touchpad was connected to a Makey-Makey® device, used by children for educational and recreational purposes. Makey-Makey® allows for any organic material to interface with a computer where contact is registered as a key press of one's choosing. Participants wore a velcro anklet which connected the Makey-Makey® device and to the metal touchpad (see Figure 2, top panel) which allowed them to interface with the MATLAB (version R.2023b) data collection program (available upon request). The program indicated whether the touchpad registered any contact during the experiment and provided guidance, such as "Participant, reach for target and touch contact when done," via a monitor facing the participant. The guidance was given as the participant advanced through the program.

Figure 1: Search Conditions

Note. The target items are indicated in red while the distractor items are blue. The left panel image shows an example of a condition varying by length, while the diameter is kept constant. The right panel image displays the 16 different conditions administered in the experiment.

Figure 2: Experimental Apparatus

Note. In the top panel image, the two containers are shown as an example for the separate frames of reference search; in the shared frame of reference search, the two containers are removed, and one container is placed on top of the velcro between the two containers. The touchpad's positioning is vital for the participant, as they tap it on the way to search in the containers. The black anklet is shown on the middle left of the table. The bottom image represents the participants' view as they enter the room. Conditions are withheld in the red cups, showing labels of their appropriate condition number (1-16). Each condition has two cups, for simpler execution in the two-container condition. The keyboard is placed at an easily accessible location for the experimenter. Participants' vision is impeded by a black poster board.

Exploring the World by Touch: Guidance in Bimanual Haptic Search

Design and Procedure

Signed consent was obtained before beginning the experiment. Subjects sat comfortably in a chair, with the sagittal midline of the body aligned with the center of the experimental setup. The experimenter began by reading the instructions, followed by the participant being asked to repeat their version of the instructions to ensure understanding. Within those instructions, it was emphasized to search for the target item as quickly and accurately as possible.

Before commencing, the experimenter established if the participant was odd-numbered or even-numbered; if the participant was odd-numbered, the experiment began with one container and swapped from one to two containers at the halfway point—after 16 trials. Even numbered participants followed the opposite procedure.

After the appropriate container and conditions were placed by the experimenter, participants used their knowledge of the instructions to begin searching by tapping the metal touchpad on their way to the Tupperware® container. In one container conditions with 6 PEX pipes, both hands would be used for search—the shared frame of reference condition. On the contrary, in two container conditions, the right hand would search in the right container consisting of 3 PEX pipes while the left hand would search in the left container consisting of the other 3 PEX pipes—the division of labor condition (see Figure 2).

After locating what was thought to be the target item, the participant removed it from the container and touched the metal touchpad again. The participant presented the target item underneath the posterboard and the experimenter delivered verbal accuracy feedback in the form of "correct" or "incorrect." The target item was red in color to help the experimenter easily distinguish it from the blue distractor items. Following feedback delivery, the participant placed the target item back into the container from which it was initially removed. The conditions were then changed by the experimenter while the participant had a brief break. This process was repeated throughout the experiment until the completion of all 32 trials. Conditions were randomized per participant. Debriefing was performed accordingly and all questions were answered.

Participants

We tested 47 UCR undergraduate students who were recruited through the Psychology Research Participation System (SONA) for course credit in the Winter 2022, Spring 2022, and Fall 2023 quarters. The study was IRB-approved. According to the Office of Diversity and Equity at UCR (Fall enrollment at a glance, 2023), the undergraduate population consisted of 33% Asian, 23.3% Hispanic, 21.6% White, 22.1% other. Gender breakdowns included 52.8% women, 44% men, and 3.2% other.

Search time data were imputed if they were less than .5 seconds, which we deemed a sign the participant double tapped the contact to start their timer and immediately stopped it. We also imputed the median time for data where the search was too long or greater than three standard deviations from the participants' mean search time. Lastly, a small amount of data was excluded due to errors in the administration of the experiment and/or equipment malfunction. Our final usable data was obtained from 45 participants which exceeded our a-priori power analysis (conducted in G*Power version 3.9.1.7), where a total sample size of 40 participants was required to compare interactions between two groups across 8 conditions with 2 measures each, to show an effect size f of 0.22 or greater.

Exploring the World by Touch: Guidance in Bimanual Haptic Search

RESULTS

Container Comparison

Figure 3 provides a comprehensive summary of the experimental findings by consolidating all of the collected data into a single omnibus figure. Inspired by Brenner and Smeets (2022), the utilization of this omnibus figure effectively combines accuracy and search times into one measure, providing a comprehensive view of the overall impact of container variation. This figure integrates accuracy—measured in proportion correct—and mean search times to generate a central metric of efficiency. This value was devised by dividing the average search times across all searches within each container condition by the proportion of correct trials (the number of correct trials divided by total trials). A two-sample t-test on the average time for correct responses revealed that the difference in the mean of the two groups was not significantly different: $t(90) = 0.765$, $p = 0.446$, 95% CI [-2.22, 5.00].

Volume Variations on Search Times

To examine the impact of target features on search times, an 8x2 analysis of variance (a repeated measures ANOVA) was conducted on the search time data for each of the eight target volumes and the two dimensions that distinguished the target from the distractors—length or diameter—in Figure 4. The results showed a significant main effect for the dimension that distinguished the target from the distractors, with mean search times differing between searching for length and searching for diameter, $F(1,736) = 31.31$, $p < .001$, $\eta^2 = .044$. However, the interaction between the target volume and the distinguishing dimension, and the main effect for the volume of the target were not appreciable, $F(7,736) = 1.06$, $p = .386$, and $F(7,736) = 1.86$, $p = .073$, respectively. The results support that the relative size ratio between the target and distractors alone was not sufficient to predict search times.

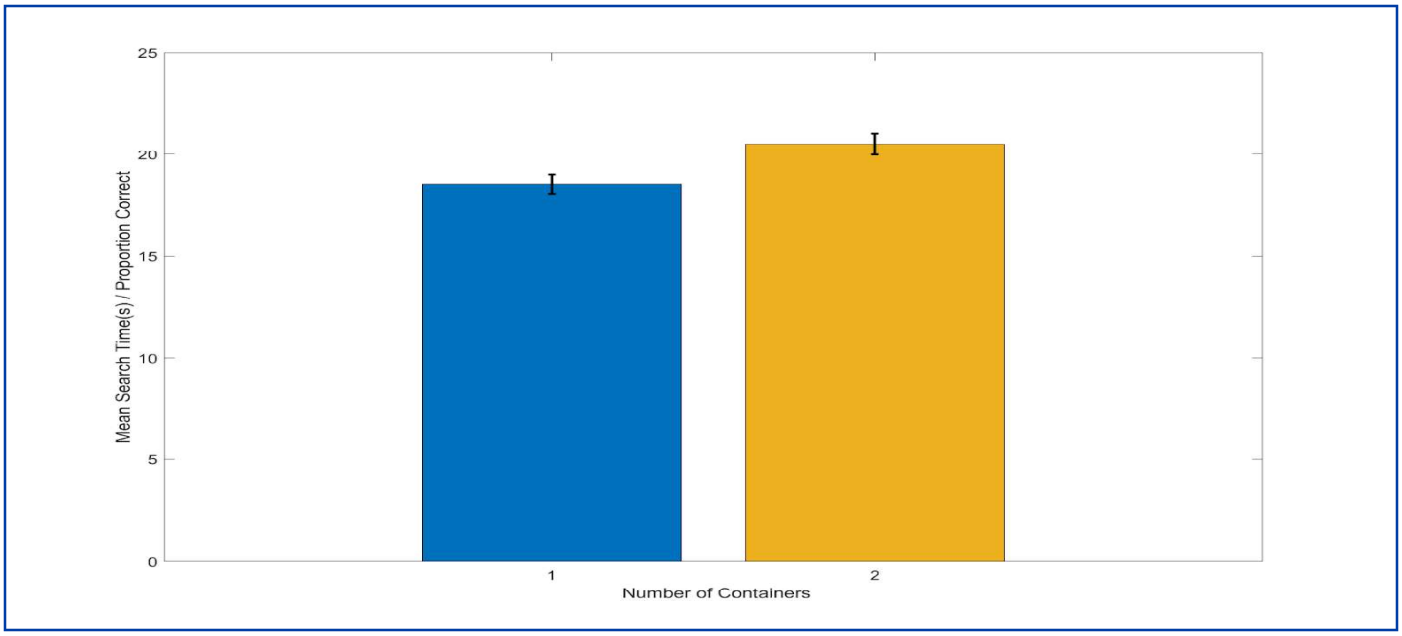

Figure 3: Grand Mean Search Times.

Note. The time for correct responses when the hands worked in a single container (blue) and when the hands worked in separate containers (gold). The time for correct response is the mean search time divided by the proportion of correct responses. The error bars represent the standard error of the mean of search times.

Exploring the World by Touch: Guidance in Bimanual Haptic Search

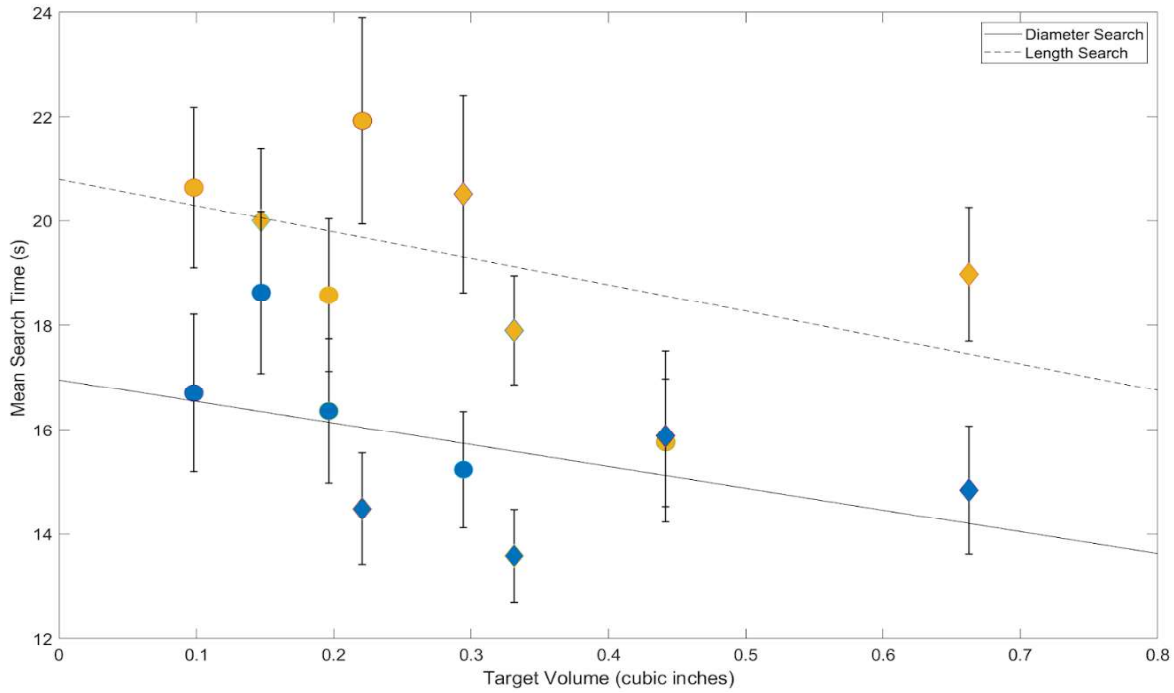

Figure 4: Mean Search Times as a Function of Volume

Note. Mean search times (±1SE) as a function of the volume of the target object when the dimension that distinguishes the target is its diameter (Blue) or length (Gold). The line represents the best-fitting regression for each of the two search types, length and diameter. The marker shape distinguishes search when the target was the smallest object (Circles) and when the target was the largest object (Diamonds).

DISCUSSION

Our study sought to shed more light on the relatively unexplored field of vision independent haptic search. We aimed to better understand some of the underlying mechanisms at play when it came to influencing the efficiency of bimanual search. We specifically focused on how the magnitude—represented by the overall volume of the targets—along with frames of reference, influenced search.

We considered two results from previous literature that were at odds with one another; haptic search efficiency was improved by either combining hands and redundancy or by dividing the hands with the shared division of labor to perform simultaneously. The question was simply, do the hands work more efficiently when together or when separated? Although the bar plot in Figure 3 initially suggested that search in a shared frame of reference was more efficient than in a single frame of reference, further statistical analysis revealed no significant difference between the two. The mean search times of one container when compared to two containers did not show any significant change in the efficiency of the search, which may indicate that the frames of reference utilized in this task simply have no effect on search times. The absence of such an effect suggests there may be additional nuances in how we direct our haptic attention which warrants further investigation. For example, separate hands may benefit from touching all the items quickly but comparison between them might be slower. Both hands working together might improve comparison, but the improvement might be offset by more slowly touching

or competing actions between the hands within the search space.

Additionally, we tested whether the relative size between the target item and distractors predicted search times. We analyzed this by varying the volume of the target while systematically varying the dimensions that distinguished the target's uniqueness. This is best seen in Figure 4, where each target volume was tested against varying size distractors, and what made the target unique was either its length difference or its diameter difference. Search times showed a gradual decrease as the volume of the target increased; however, the dimension that distinguished the target was of significant importance to determining the time spent searching. An additive effect was observed between diameter and length searches, where the feature of length continuously resulted in longer search times when compared to diameter searches for the same target volume. This suggests that haptics is guided not just by the physical dimensions of the objects, but also by the feature in which those dimensions differed. The same volume-cylinder target was either more or less difficult depending on whether it differed from the distractor with respect to diameter or length.

LIMITATIONS & FUTURE DIRECTION

Although this study has provided valuable insights into the field of haptic search, it is important to acknowledge that there were several limitations. The study aimed to optimize ecological validity with the use of common materials, a naturalistic environment, and realistic search conditions. However, our experiment may not have fully captured and reflected the complexities of a real-world haptic search task and the items we often search for. In addition to that, the explored variables consisted of magnitude and frames of reference, which may exclude additional factors contributing to efficient searches, such as multi-sensory facilitation. Lastly, the population utilized for this study was solely composed of undergraduate students, limiting generalization to other ages and demographics.

Future research could delve into the positioning of the participant while searching. Positioning the search containers in non-standard positions regarding the body, such as a more leftward position or spreading the two container conditions further apart, should be pursued. This could provide more insight into how the body positioning and varying locations of search may influence the frame of reference and haptic efficiency.

CONCLUSION

By elucidating how different factors influence haptic attention and search, our findings contribute to the growing body of literature on haptic search and have implications for various fields, including prosthetics and human-computer interaction technologies. With further investigation into the dynamics of haptic attention and how frame of reference influences search, future research could advance our understanding of haptic perception and inform the development of these assistive technologies for individuals with sensory impairments. Given this, it is important to acknowledge that this field of research has received relatively little attention thus far, emphasizing the need for further investigation.

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Exploring the World by Touch: Guidance in Bimanual Haptic Search

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Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

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ABSTRACT

In biological studies in vitro and in vivo, techniques involving Förster resonance energy transfer (FRET) and FRET quantification use the interaction of CyPet-SUMO1 and its E2 ligase, YPet-Ubc9, to determine the dissociation constant (K_D). Dipole-dipole resonance interactions, where energy transfers from an excited donor to an acceptor chromophore, allow the detection of molecular interactions to elucidate protein interactions in many regulatory cascades spanning signal transduction, medical diagnostics, and optical imaging. This study aims to explore how protein-protein interactions are affected by the crowded environment typically found within cells using FRET signals. An in vitro assay using a 96-well plate was conducted using varying concentrations of bovine serum albumin (BSA) to simulate crowded conditions and determine their effect on K_D values. FRET measurements were conducted in a solution phase to mimic the protein interaction affinity in living cells. In contrast, other K_D measurement methods such as radio-labeled ligand binding assay, surface plasmon resonance (SPR), or isothermal titration calorimetry (ITC) require extensive preparation or orientation on solid surfaces, making them less representative for such assessments. Emission wavelengths from CyPet-SUMO1 (414 nm to 475 nm) and YPet-Ubc9 (475 nm to 530 nm) were obtained to determine fluorescence signals along with K_D. A comparison between protein interactions in crowded and uncrowded settings was made with varying K_D value results. This investigation provides insights into protein interactions and cellular crowding, with potential implications for pharmaceuticals, bioseparations processes, and drug discovery targeting protein-protein interactions.

KEYWORDS: protein-protein interactions, K_D, qFRET, FRET signals, bovine serum albumin (BSA), crowded proteins, SUMOylation

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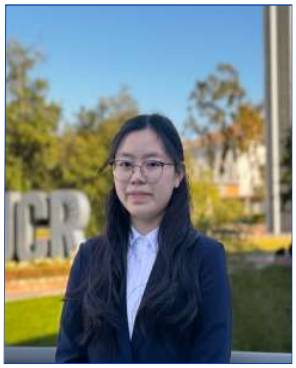

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Mandy Hsieh is a third-year Bioengineering major with a minor in Data Science. She leads the Power Team in AIChE's ChemE Car Competition and is passionate about regenerative medicine, tissue engineering, and biophysics. A part of the B2K Group under Dr. Victor G. J. Rodgers, Mandy also contributes to the chemical synthesis/ bioprinting team led by Dr. Iman Noshadi in the Innovative Biomaterials Laboratory, receiving a mini-grant award. Mandy aspires to earn a Ph.D. in chemical engineering or bioengineering.

Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

INTRODUCTION

Traditional approaches to mimic cellular environments for studying enzymatic processes often involve observing reactions in dilute buffers. However, the intracellular environment of living cells is densely crowded with macromolecules such as proteins, nucleic acids, ribonucleoproteins, polysaccharides, and metabolites. This combination of concentrated multicomponent solutes is known as crowding. Crowding has been found to significantly impact enzymatic activities both in vitro and in vivo, thereby challenging the validity of these representations.[1] Through the use of quantitative Förster resonance energy transfer (qFRET) imaging, an investigation is conducted to determine whether a crowded environment influences the dissociation constant (K_D) values in experimental settings. The protein, bovine serum albumin (BSA), is used as a crowding agent, and qFRET technology is utilized to interpret FRET signals and quantify binding kinetics in crowded environments. Understanding the related kinetics can provide valuable insights into protein interactions in vivo, contributing to a better understanding of cellular processes and potentially guiding drug discovery efforts targeting protein-protein interactions.

Crowding agents are employed to simulate the densely packed environment, mimicking the crowded conditions of cell interiors. This affects molecular interactions and can influence various biochemical processes, as crowded conditions are shown to alter binding affinities. In this study, BSA, whose pH closely resembles that of a cellular environment, acts as a crowding agent to replicate the crowded conditions within cells. Albumin, a protein present in BSA, contains histidine residues, and its functional group of imidazole permits effective protonation and deprotonation based on its surrounding environment. This property makes serum albumin, including BSA, an excellent buffer and a suitable candidate for maintaining protein behaviors and properties without alteration. However, other inert crowding agents like polyethylene glycol (PEG), dextran, and Ficoll can also be effective alternatives as these agents increase viscosity, reducing free space, to simulate cellular environments.[1, 2]

This work focuses on the reversible reaction (SUMOylation) between a small ubiquitin-like modifier (SUMO) and its E2 ligase, Ubc9 to elucidate how crowding affects protein-protein interactions. SUMOylation is a post-translational modification (PTM) that involves a multistep enzymatic cascade reaction that results in peptide activation and substrate conjugation.[3] Other PTMs include ubiquitin (Ub) and ubiquitin-like (Ubl) proteins that regulate protein activities and half-lives in eukaryotes.[4] Unlike some interactions, this interaction does not require an activation cascade to begin due to its inherent nonvalent affinity.[3] The engineered fluorescent proteins, CyPet-SUMO1 and YPet-Ubc9 pairs, will help determine the K_D of the SUMO1 and Ubc9 interactions. The dissociation constant in this case is given as $K_D = \frac{[A][B]}{[AB]}$, where [A] is CyPet-SUMO1, [B] is YPet-Ubc9, and [AB] is the concentration of the complex in equilibrium. FRET signal analysis and K_D measurement are made from FRET responses, allowing for the direct determination of K_D from the FRET signal. The objective of the study is to compare the binding affinity of CyPet-SUMO1 and YPet-Ubc9 in various crowding conditions, using BSA as the crowding agent. With this, K_D represents the ratio of the concentrations of free and bound proteins. A one-way ANOVA test will be performed to determine the statistical significance among the means between crowded and non-crowded environments.

Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

METHODOLOGY

Experimental Setup for Finding Absolute FRET (EmFRET) Signals

A Costar® black and clear-bottom 96 well-plate was utilized, and a solution containing 350 g/L of BSA yielded a measured pH reading of 7.94. The BSA solution comprised equal parts of artificial cerebrospinal fluid (aCSF) to a 350 g/L, pH 7.4 BSA solution. The artificial cerebrospinal fluid (aCSF) salt solution contained 0.214 g of dibasic sodium phosphate and 0.027 g of monobasic sodium phosphate dissolved in 500 mL of pyrogen-free sterile water. The 350 g/L BSA solution was diluted with aCSF salt solution to acquire different concentrations of FRET readings in the 96 well-plate, with 200 µL per well, repeated three times with a total of 600 µL per well and an extra 200 µL to account for pipetting errors. Fluorescent proteins, CyPet-SUMO1, with a concentration of 55.50 µM, and its E2 ligase, YPet-Ubc9, with a concentration of 61.1 µM, were obtained through protein purification. A calculation was performed using 1 µM for CyPet and YPet to determine the required µL needed to achieve the correct concentrations of BSA, ensuring that each well contains 200µL of total suspended solution. By diluting 350 g/L of BSA with aCSF salt solution, various concentrations of BSA ranging from 70-100 g/L were achieved for low-crowding, while 250-300 g/L of BSA were obtained for high-crowding. Because of the high viscosity of the solutions, the pipette tips were trimmed to ensure precise measurements for high BSA concentrations. EmFRET signals were determined using a spectrophotometer (SpectraMax M3™, Molecular Devices, San Jose, CA).

Additionally, BSA was measured separately from the fluorescent proteins due to its slight yellow coloration. BSA's signal readings were subtracted from the tagged protein emission wavelengths of CyPet-SUMO1 at 414 nm to 475 nm, YPet-Ubc9 at 475 nm to 530 nm, and the total emission wavelengths of both fluorescent proteins from 414 nm to 530 nm. This subtraction ensured accurate protein affinity measurements and eliminated potential interference from other colorations and wavelengths on the EmFRET readings.

After pipetting all necessary elements to achieve varying crowding scenarios at different BSA concentrations with 200 µL per well, the proteins were incubated at 37°C to mimic a cellular environment for about 10-15 minutes. They were centrifuged and mixed until homogeneous, and the well-plate was transferred to a plate reader where the software, SoftMaxPro (version 6.1, Molecular Devices, San Jose, CA) was employed to generate EmFRET readings, crucial for determining K_D. There, a selective comparison between the high-crowding and low-crowding scenarios was made and a decision was selected for K_D calculations. K_D measurements were conducted under crowding conditions of 0 g/L of BSA for non-crowding, 95 g/L of BSA for low-crowding, and 290 g/L of BSA for high-crowding.

K_D Determination

For K_D determination, the same setup was employed, including the use of a black and clear-bottom 96 well-plate, 350 g/L pH 7.4 BSA solution, aCSF salt solution, fluorescent proteins at consistent molar concentrations (CyPet-SUMO1 & YPet-Ubc9), the incubation step, and the software, SoftMaxPro. K_D values were calculated using Prism 5 (GraphPad Software, La Jolla, CA) for non-crowding (no BSA), low-crowding (95 g/L of BSA), and high-crowding (290 g/L of BSA) scenarios, with three replicates conducted to ensure the accuracy of protein affinity measurements. In both the setups with 95 g/L and 290 g/L of BSA, the molarity of YPet-Ubc9 varied with the volume of the aCSF solution to maintain the same BSA concentration, while CyPet-SUMO1 remained constant. From this, 14 K_D incremental steps with different substrate values

Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

(μM) were used, resulting in 15 total YPet-Ubc9 (μM) EmFRET signals including the initial step at 0 μM. The alpha (α) and beta (β) values were calculated once, where alpha values were measured with CyPet-SUMO1 without YPet-Ubc9 (1 starting step), and beta was calculated with only YPet-Ubc9 present, without CyPet-SUMO1 (13 incremental steps). The EmFRET signal is determined from

$$Em_{FRET} = (FL_{DA}) - \alpha(FL_{DD}) - \beta(FL_{AA}), \quad (1)$$

where FL_{DA} is the total fluorescence emission at the acceptor wavelength when excited at the donor excitation wavelength, FL_{DD} is the fluorescence emission at the donor wavelength when excited at the donor excitation wavelength, and FL_{AA} is the fluorescence emission at the acceptor wavelength when excited at the acceptor excitation wavelength.[3] These incremental steps involved altering the volume while maintaining the same concentration for three experiments: high-crowding conditions with 290 g/L of BSA, low-crowding conditions with 95 g/L of BSA, and non-crowding conditions with 0 g/L of BSA. While keeping the concentration of one reactant constant and changing its volume, an observation of how the equilibrium shifts with respect to its changes in concentration was made with its K_D value. This offered valuable insights into the strength of the interaction between the protein molecules and aided in determining K_D. After obtaining the necessary data from SoftMaxPro and Prism 5, the following equations[3] were regressed to find K_D:

$$Em_{FRET} = Em_{FRET_{max}}\left(1 - \frac{2K_D}{X-A+K_D+\sqrt{2(X-A-K_D)+4XK_D}}\right) \quad (2)$$

where A is the fixed concentration of CyPet-SUMO1, X is the different concentrations of YPet-Ubc9, and $Em_{FRETmax}$ is the maximum Em_{FRET} signal.

RESULTS AND DISCUSSION

A concentration of 290 g/L of BSA was selected to characterize high-crowded conditions for K_D determination. Conversely, 95 g/L of BSA is chosen to represent low-crowded conditions. Tables 1-3 summarize the EmFRET signals in relative fluorescence units (RFU) obtained.

BSA (g/L)	Trial 1	Trial 2	Trial 3
300	2343 ± 65	2406 ± 39	2759 ± 105
290	2503 ± 24	2524 ± 15	2657 ± 39
280	2584 ± 3	2506 ± 29	2642 ± 26
270	2795 ± 48	2584 ± 38	2655 ± 9
260	2884 ± 26	2932 ± 6	3025 ± 32
250	3007 ± 110	2575 ± 67	2634 ± 43
aCSF	6413 ± 239	7768 ± 314	6812 ± 76

Table 1. Three trials were conducted for each test to minimize inaccuracies, using BSA concentrations ranging from 250 g/L to 300 g/L to create a highly crowded environment.

BSA (g/L)	Trial 1	Trial 2	Trial 3
100	5443 ± 121	5402 ± 104	4598 ± 224
95	4473 ± 14	4433 ± 3	4413 ± 11
90	3803 ± 8	3796 ± 5	3751 ± 13
85	4108 ± 64	3958 ± 3	3788 ± 67
80	4009 ± 30	4039 ± 43	3754 ± 73
75	4088 ± 22	4205 ± 26	4133 ± 4
70	4433 ± 61	3840 ± 181	4576 ± 120
aCSF	6187 ± 95	6074 ± 49	5599 ± 145

Table 2. Three trials were conducted for each test to minimize inaccuracies, using BSA concentrations ranging from 70 g/L to 100 g/L to create a low-crowded environment.

Additionally, a control group with no crowding agent (BSA) present is collected in both high-crowded and low-crowded environments, and their values are compared. This simulates the condition of cells in a non-crowded environment, providing a basis for comparison in the study of protein-protein interactions in crowded environments.

Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

aCSF with CyPet/YPet	Trial 1	Trial 2	Trial 3
aCSF in Table 1	6413 ± 239	7768 ± 314	6812 ± 76
aCSF in Table 2	6187 ± 95	6074 ± 49	5599 ± 145

Table 3. A non-crowded environment is demonstrated by excluding BSA, using only aCSF salt solution from Table 1 and Table 2's experiment values with the fluorescent proteins.

In general, all EmFRET signals follow the same shape of the depicted curve in Figure 1 with CyPet-SUMO1 and YPet-Ubc9 fluorescent-tagged proteins. The fluorescent emission at the donor, CyPet, ranges from 414 nm to 475 nm, while the fluorescent emission at the acceptor, Ypet, ranges from 475 nm to 530 nm. CyPet is quenching, or losing energy, and Ypet is excited, gaining energy.[3] Once the EmFRET signals are recorded, K_D and EmFRETmax can simultaneously be determined in Equation 1, where a nonlinear regression is used for each set of experiments with different total concentrations of YPet-Ubc9 (μL). The K_D of CyPet-SUMO1 and YPet-Ubc9 were then plotted using the non-linear regression of EmFRET (RFU) vs. $[YPet\text{-}Ubc9]_{total}$ (μM), and the resulting K_D values are shown in Figure 2.

Figure 2 summarizes the K_D values obtained. The experiments revealed that binding in crowded environments is reduced due to crowding as evidenced by the respective increase in K_D values. The ANOVA test in Figure 2 yielded P values of 0.0123 for non-crowding vs. low-crowding, and 0.0340 for non-crowding vs. high-crowding, both values falling below 0.05. This suggests sufficient evidence to reject the null hypothesis and conclude significant differences exist between non-crowded and crowded environments. However, the Brown-Forsythe test comparing high-crowding and low-crowding conditions, yielded a P value of 0.3891, which exceeds the significance threshold of 0.05. This indicates that there is no significant difference between the high and low crowding conditions. In addition, altering the concentration of the crowding agent, BSA, from 95 g/L to 290 g/L does not affect the K_D results, as revealed by the comparison between the two, which shows no significance. However, a notable distinction emerges between a non-crowded environment (the absence of BSA) with the crowded environments in both low and high-crowded settings.

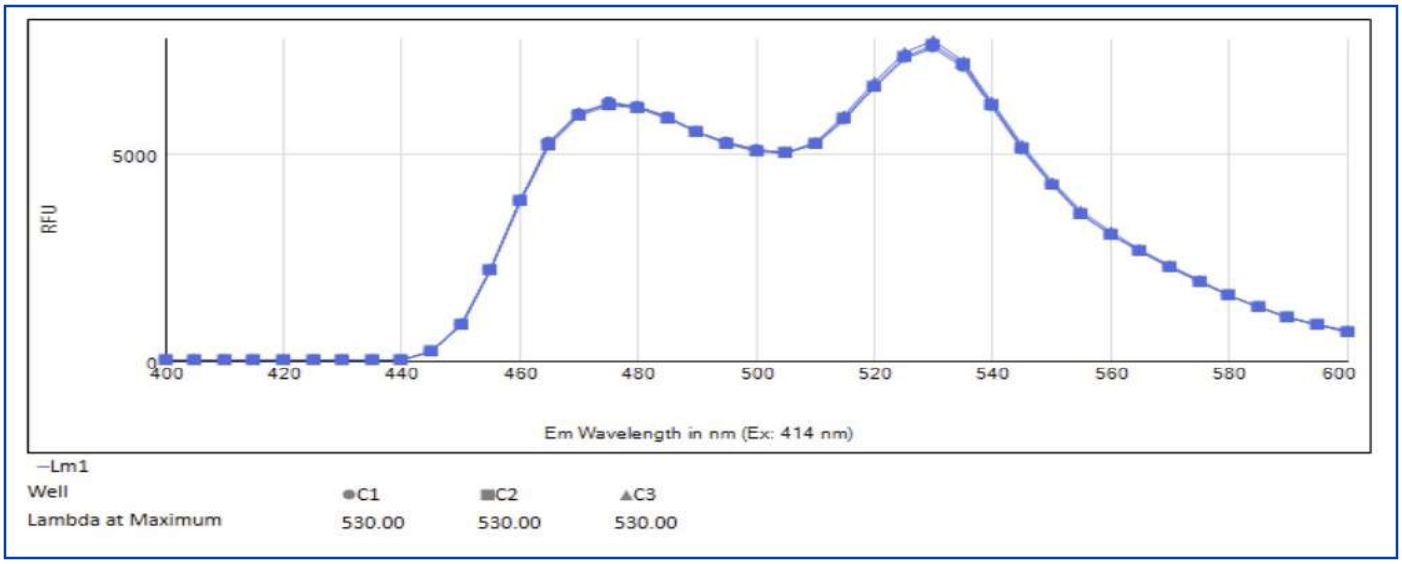

Figure 1. The general trend for FRET Emission Signals (EmFRET) in RFU Reading Peaks for 290 g/L of BSA in Table 1. The plots represent the three trials run in the well plate.

Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

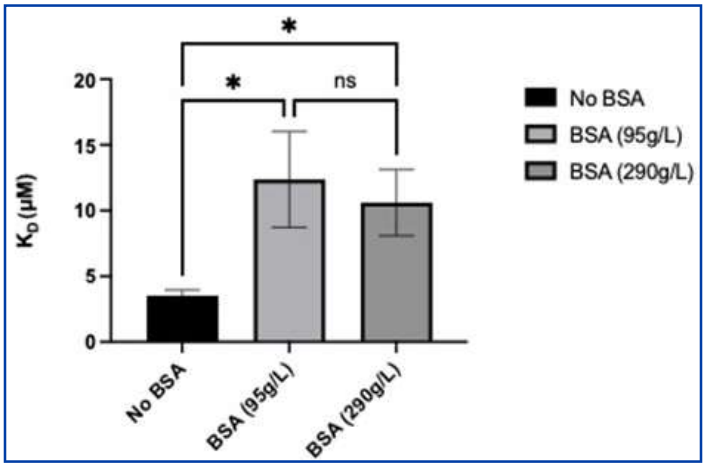

Figure 2. Resulting K_D values. The presence of the crowding agent, BSA, increases K_D by approximately a factor of three. Further increasing BSA concentration from 95 g/L to 290 g/L does not statistically change K_D. Error analysis was determined using one-way ANOVA. The * indicates $P < 0.05$. ns indicates no significant difference.

CONCLUSION

Using the qFRET assay, we determined K_D for the dissociation equilibrium constant for CyPet and YPet with SUMO1 proteins under crowded conditions.[3, 5] A higher K_D value suggests weaker binding, whereas a lower K_D value suggests stronger binding. A crowding concentration of BSA resulted in K_D values of 3.5 ± 0.5 μM, 12.4 ± 3.7 μM, and 10.6 ± 2.5 μM for 0, 95, and 290 g/L BSA, respectively. The data indicates that the crowding agent elevates K_D by approximately a factor of three, underscoring the significant impact of crowding on the dissociation constant, K_D. This indicates that in crowded conditions, the presence of other molecules or crowders reduces the affinity between proteins, leading to weaker binding compared to non-crowded environments or diluted solutions. This conflicts with traditional approaches to mimic cellular environments for studying enzymatic processes using dilute buffer solutions. Figure 2 also depicts that the EmFRET signals appear noisier at 290 g/L of BSA than in other concentrations, suggesting that crowding may interfere with the spectrometer signals.

Future investigations will focus on identifying the point at which crowded conditions are established and their subsequent impact on K_D values. Alternative crowding agents such as human serum albumin (HSA), polyethylene glycol (PEG), dextran, or Ficoll could be used in future studies to assess the significance of crowding agent compositions on K_D and better simulate environments in various cell types. This includes determining different crowding effects, for example, in cardiomyocytes or osteoclasts, particularly for reversible reactions in cells. Further reassessment of K_D values determined in dilute solutions can be conducted and compared with the K_D values in crowded conditions. Additional studies include measuring osmotic pressures in protein solutions, as previous research established that crowding agents impact osmotic pressure in cellular environments.[7-12] These studies can provide insights into how proteins behave in different environments, and because cells have high osmotic pressure, the pressure in the presence of crowding agents can be used to measure the value of K_D. This understanding could aid in designing controlled-release systems and predicting drug behavior in physiological environments, with potential implications for pharmaceuticals, bioseparations processes, and drug discovery targeting protein-protein interactions.

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Interpreting Crowding Effects on FRET Signals for Protein Kinetics Analysis

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The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

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ABSTRACT

Despite increased institutional awareness toward diversity, equity, and inclusion on college campuses, LGBTQ+ students–especially QTPoC students–continue to experience disproportionate mental and behavioral health burdens and food insecurity compared to their cisgender and heterosexual peers. To address this issue, QTPOC (Queer and Trans People of Color), a student organization that supports students at the intersection of oppression based on race, gender, and/or sexuality on campus, sought to create a programming model which integrates educational elements with incentives to attend events. We aim to understand how the mental and behavioral burden for LGBTQ+ student populations can be improved, with a specific focus on QTPoC. Specifically, does implementing our programming strategy of blending food provision with peer-to-peer interaction improve the overall well-being of QTPoC? We aim to assess our programming's impact by understanding and addressing factors behind mental health disparities. We sampled college students who attended the eight QTPOC events held during the 2023-2024 Winter Quarter: n= 211; ~18% QTPoC. We recruited QTPoC students to complete pre- and post-event surveys to assess attendance, motivation, well-being, and awareness of campus resources regarding both food insecurity and sexual and gender health. We found that there was increased knowledge and well-being among QTPoC students who attended QTPOC events. We also found that offering hot food significantly contributed to positive experiences, fostered community building, and alleviated concerns regarding food insecurity. We conclude that our programming model effectively combats mental health disparities and food insecurity among QTPoC students.

KEYWORDS: LGBTQ+, QTPOC, food insecurity, Mental Health, College Campus

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Abtin Shafie is a third-year Bioengineering major studying QTPoC populations under Dr. Nicholas Napolio. His research is funded by UCR's Basic Needs Department and LGBT Resource Center. He is a part of several student organizations and a student staff at UCR's LGBT Resource Center. Abtin is a recipient of the Undergraduate Student Leadership LAMBDA Award and the UCR LGBTQIA Student Leadership Scholarship.

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

INTRODUCTION

We aim to increase information about how QTPoC-serving student organizations can best serve QTPoC within a college setting. QTPoC represents an intersectional identity, encompassing multiple dimensions of oppression based on race, gender, and sexuality (Duran, 2019), thus we believe a multifaceted approach is needed to effectively address QTPoC needs. Within this paper, we will refer to QTPOC as the student organization and QTPoC as individuals identifying as queer and trans people of color. QTPOC events focus on mental well-being, LGBTQ+ knowledge, and community building. Does our programming model of integrating food with peer-to-peer support improve the overall well-being of QTPoC? Specifically, we aim to determine if food incentives attract QTPoC to QTPOC events. QTPOC events create a third space where attendees can benefit from engaging in peer-to-peer support and gain information about on-campus resources. We recognize that improving well-being can entail different approaches based on an individual's needs.

Peer-to-Peer Support

As a student organization, QTPOC uniquely serves QTPoC students by offering social and emotional support grounded in shared lived experiences. Peer support has been recognized as an effective strategy for enhancing mental health outcomes among young adults (Richard, 2022). Through QTPOC, individuals have access to a network of peers who may exchange advice on how to effectively navigate life as part of the QTPoC community. We aim to enhance our events by facilitating peer-to-peer support through direct engagement with our members and fostering open conversations to promote positive mental health outcomes.

Shared Lived Experience

Creating an atmosphere enriched with shared lived experiences is critical amongst LGBTQ+ folks. It provides validation and understanding by allowing individuals to connect with others who have faced similar challenges and discrimination (Balsam & Mohr, 2007). This validation could help affirm one's identity and contribute to positive mental health outcomes by reducing feelings of isolation and alienation. Additionally, shared lived experience fosters a sense of belonging within the LGBTQ+ community (Frost & Meyer, 2009). Shared lived experiences often involve peer-to-peer interactions, where peers can serve as on-campus resources given the variety of knowledge shared. This support network is invaluable for promoting mental health and well-being among LGBTQ+ individuals (McCurdy, 2023).

Third Space

The idea of a "third space" was first popularized by Ray Oldenburg in his book The Great Good Place: Cafes, Coffee Shops, Bookstores, Bars, Hair Salons, and Other Hangouts at the Heart of a Community (1989). Oldenburg states that third spaces play a crucial role in building social connections outside of the home (first space) and workplace (second space) (Oldenburg, 1989). Third spaces play a vital role in the lives of LGBTQ+ students by providing environments where they can authentically express themselves, connect with like-minded peers, and find support. These spaces, which may include LGBTQ+ resource centers or student organizations, may offer a sense of belonging and affirmation (Rankin et al., 2010).

QTPOC contributes to third spaces for LGBTQ+ students by creating an inclusive environment designed by the needs and experiences of QTPoC and LGBTQ+ peers. As a student-run organization, QTPOC is uniquely positioned to address the specific needs and experiences of QTPoC individuals on campus because it is ever-evolving to serve the needs of its

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

members, meaning that its programming remains relevant and effective in promoting the well-being and empowerment of QTPoC individuals. QTPOC's presence, as well as its events, create a third space sends a powerful message of visibility and representation, affirming the diverse identities and experiences within the LGBTQ+ community.

The Impact of LGBTQ+ Clubs on Campus

LGBTQ+ clubs and organizations have a profound impact on LGBTQ+ individuals, especially on college campuses by providing essential support, community, and resources that aid in mental wellness (Rankin et al., 2010). These groups serve as safe spaces where LGBTQ+ students can connect with peers who hold similar identities and experiences, fostering a sense of belonging and acceptance. While the college or university experience is typically marked by growth and learning, some students encounter distinct challenges due to their sexual orientation or gender identity (Renn & Bilodeau, 2005).

Additionally, trans- and queer-identifying students may feel negatively about campus resources because they perceive them as inadequate or unsupportive of their identities (Garvey & Rankin, 2015). Student-run organizations can offer support by selecting topics of relevance and determining the most effective ways for peers to support one another. Our study, conducted through QTPOC, emphasizes the importance of student-led initiatives in addressing the unique challenges faced by LGBTQ+ students and creating supportive environments on college campuses. Participation in LGBTQ+ organizations provides support and community, as well as empowers individuals to advocate for their own rights and those of their peers, ultimately increasing visibility on campus.

Mental Well-Being

Stigma, prejudice, and discrimination against LGBTQ+ individuals often result in challenges with self-acceptance, which is a recognized risk factor for adverse mental health outcomes (Camp, 2020). Along with this, sparse literature on mental health promotion emphasizes the significance of adopting strength-based community approaches to promote the well-being of LGBTQ+ individuals (Ceatha, 2019).

A study highlighted the positive correlation between belongingness to the institution, perceived social adjustment, and achievement motivation to well-being (Sotardi et al., 2022). This emphasizes how factors like belongingness, social adjustment, and academic self-efficacy intersect with the well-being of LGBTQ+ students in a college setting. It's crucial to acknowledge that addressing mental well-being is multifaceted, thus incorporating elements such as peer-to-peer support, shared lived experiences and third spaces is critical to provide connection, validation, and support.

Food Insecurity

Recognizing the interconnected nature of mental health and food security highlights the importance of considering food insecurity as a factor in the overall well-being of the LGBTQ+ community. LGBTQ+ individuals are more likely to experience food insecurity compared to their heterosexual and cisgender counterparts (Ferrero, 2023). Eating disorders, disordered eating behaviors, and body dissatisfaction are common among sexual and gender minority populations, potentially exacerbated by minority stress and discrimination (Nagata et al., 2020). By providing food at events, QTPOC addresses immediate food insecurity and creates opportunities for individuals to connect. These events serve as safe spaces where attendees can share their experiences, discuss challenges they may be facing, and access resources and support networks. Additionally, sharing a meal fosters a sense

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

of community and belonging, which can help alleviate feelings of isolation and strengthen social connections. Addressing food insecurity among the LGBTQ+ population requires a multi-faceted approach that addresses systemic inequalities and provides targeted support and resources.

Research Question

Our organization integrates peer-to-peer support, shared lived experience, and the concept of a third space within its events, creating a supportive environment for QTPoC students and aiding in their mental well-being. Additionally, we introduced on-campus resources that students can use to fight food insecurity and improve mental well-being. By providing food at events, QTPOC incentivizes attendance and addresses food insecurity while fostering connections among attendees. Our main research question seeks to quantitatively and qualitatively analyze if integrating food with peer-to-peer support improves the overall well-being of QTPoC students.

METHODOLOGY

Through pre- and post-event surveys, we assess the impact of our events on attendees' experiences and knowledge. Our events are focused on promoting community building, increasing awareness of on-campus resources, and addressing food insecurity by providing hot meals. We consistently provide food at events to ensure reliability and encourage ongoing engagement within the community. All events took place at the University of California, Riverside, a public university: 100% of the participant data was obtained from QTPOC events, which are open to all UCR students, staff, and faculty. The sample size totaled 211 participants over eight QTPOC events held during the 2023-2024 Winter Quarter. We reviewed all responses solely from QTPoC-identifying individuals over 18 who completed the pre- and post-survey. This totaled to 39 participants. A t-test was used to analyze the average pre- and post-survey ratings of mental health. All P values < 0.05 were taken as statistically significant.

Procedure

Surveys were created before the event. At the event's start, we described the event and the QTPOC Project to explain our research goals and purpose for administering surveys. We then distributed the pre-event survey through a QR code via projector screen. The first page of the survey was the consent form, and the following pages had questions to collect data. At the event's end, we distributed the post-event survey through a QR code via projector screen. We used our UCR Google account to administer the survey through Google Forms.

The pre-survey asked various questions about the respondents' demographic/background, mental health-related questions, knowledge regarding on-campus resources, motivation for attending, and event-specific questions. The post-event survey asked mental health-related questions, knowledge regarding on-campus resources, how inclusive the space was, how the provision of food impacted the event experience, and how enjoyable the event was. The surveys took approximately 5 minutes each to complete. There was a "prefer not to answer" option on the survey. Participants were informed verbally and through the consent form that they may contact the study personnel at any time should they want to withdraw from this study.

Hypothesis

We expect that QTPOC will positively impact student well-being and knowledge about on-campus resources. QTPOC takes a multifaceted approach by serving as a third space for QTPoC, offering peer-to-peer support, and addressing mental well-being, with an emphasis on food insecurity.

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

RESULTS

The following section discusses the results of our survey, both quantitative and qualitative data, and is represented by various figures. The quantitative and qualitative data represented in Figures 1 through 6 was collected across eight QTPOC events during the Winter quarter and span 39 individuals who self-identified as QTPoC on the surveys distributed.

Data Analysis

We wanted to observe if QTPOC events impacted student well-being, combated food insecurity, and provided knowledge regarding on-campus food insecurity resources. We collected both qualitative and quantitative data in our surveys. The qualitative data provides backing for our quantitative data by clarifying the role that the programming model plays in impacting attendees' mental well-being.

Please note that there is a possibility that the same QTPoC participants were present at multiple events. Additionally, Figures 2 through 4 are based on survey questions with a "select all that apply" option, and participants who selected multiple options had their responses represented by their own category.

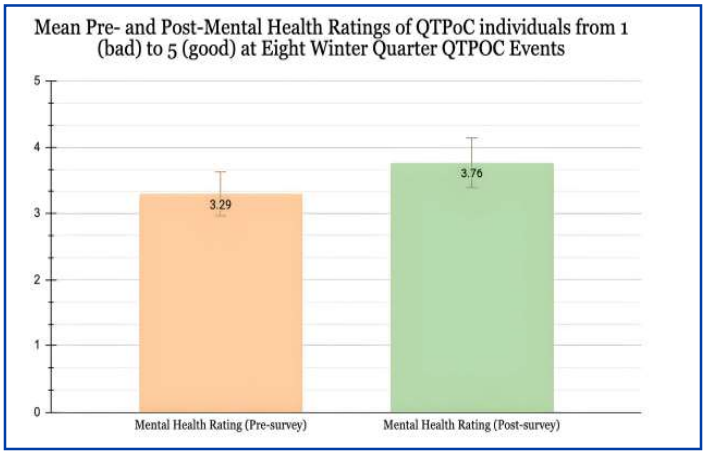

Figure 1: Average Pre- and Post-Survey Mental Health Ratings of QTPoC at QTPOC Events

Bar graph displays the average pre-survey mental health rating in orange and the post-survey mental health rating in green. Average mental health and the start and end of events over eight QTPOC events in the winter quarter.

Quantitative

Figure 1 depicts the average pre-survey mental health compared to the post-survey mental health across 8 QTPOC events spanning 39 self-identifying QTPoC as found in the data. QTPoC rated their mental health at approximately 3.29 on a scale of 1 (bad) to 5 (good) in the pre-event survey. At the end of events, QTPoC reported their mental health was at approximately a 3.76. The standard deviation for the pre-survey mental health data was 1.06 and the post-survey mental health data had a standard deviation of 0.91. Additionally, the variance for the pre-survey data is approximately 1.130 while the post-survey mental health data is approximately 0.834. A T-test was performed on the pre-survey and post-survey mental health data, which showed statistical significance with a value of 0.041. We observed that QTPoC mental health improved by the end of QTPOC events by approximately 14.3%.

Figure 2a depicts the racial/ethnic demographic of the participants who self-identified as QTPoC in the pre-surveys across eight QTPOC events. From the pie chart, we see that the largest racial/ethnic group of QTPoC at QTPOC events was East Asian (25.6%) followed by Hispanic, Latinx, or Spanish (20.5%). This is representative of the overall UCR population which is largely "Chicanx/Latinx" and "Asian" (Institutional Research, 2023). South Asian was the third largest racial/ethnic group of QTPoC at QTPOC events. Racially mixed identifying QTPoC were displayed in a new category as seen in the pie chart. Figure 2b depicts the sexual orientation of QTPoC attendees across eight QTPOC events. 25% of QTPoC self-identified as gay and another 25% identified as bisexual. This roughly reflects the overall student population at UCR. After heterosexual, "Bisexual" and "Gay/Lesbian" are the two highest percentages (UCR Student Affairs, 2021).

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

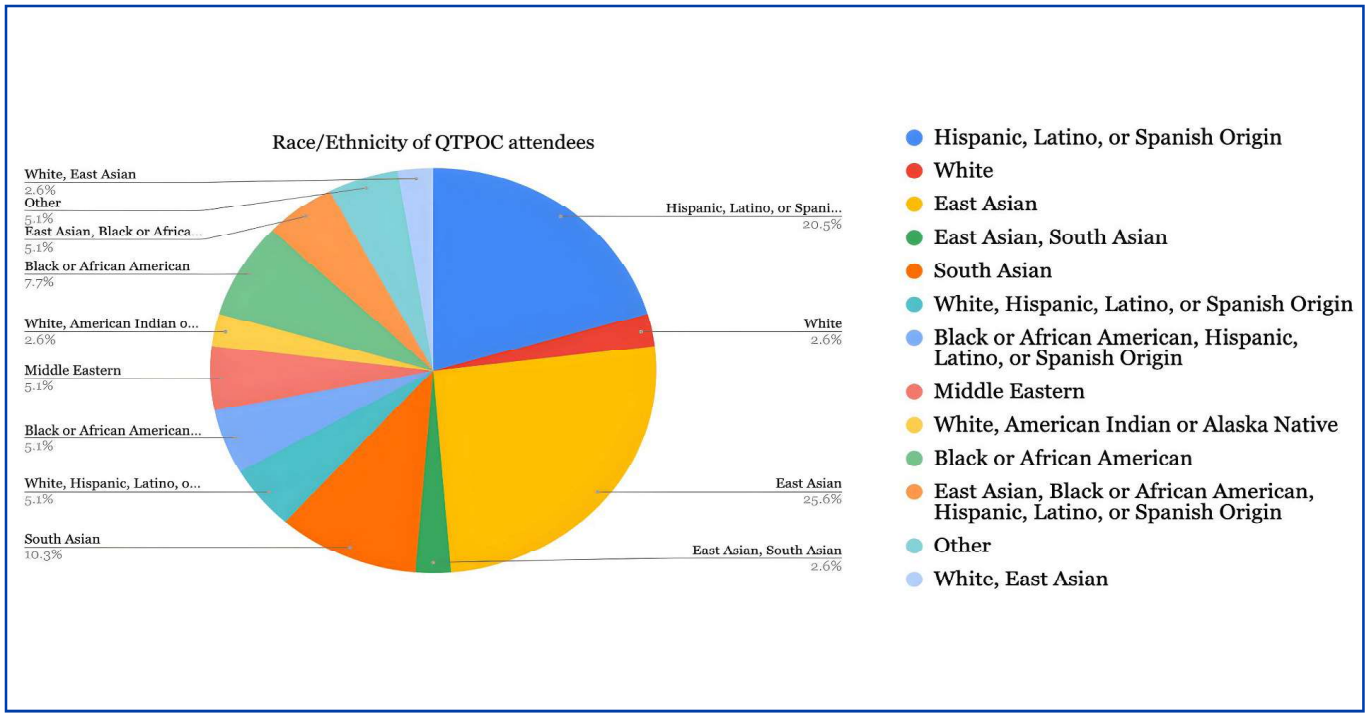

Figure 2a: Pie Chart of Race/Ethnicity of QTPOC attendees

A pie-chart graph of the different races/ethnicities of QTPOC Attendees at the eight events.

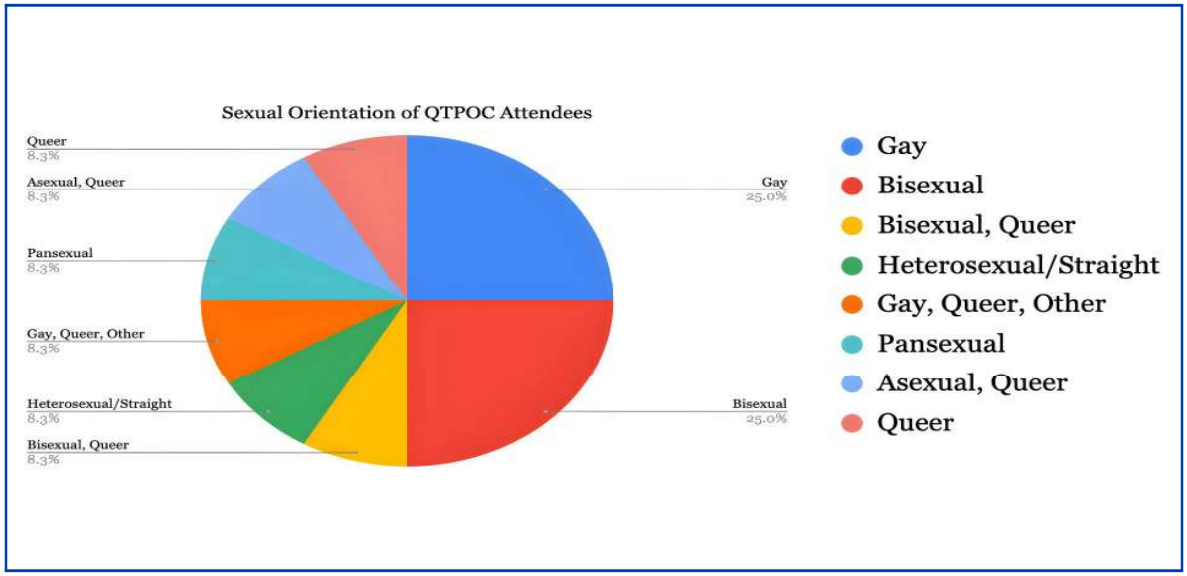

Figure 2b: Sexual Orientation of QTPOC Attendees

A pie-chart graph of the different sexual orientations of QTPOC attendees at the eight events.

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

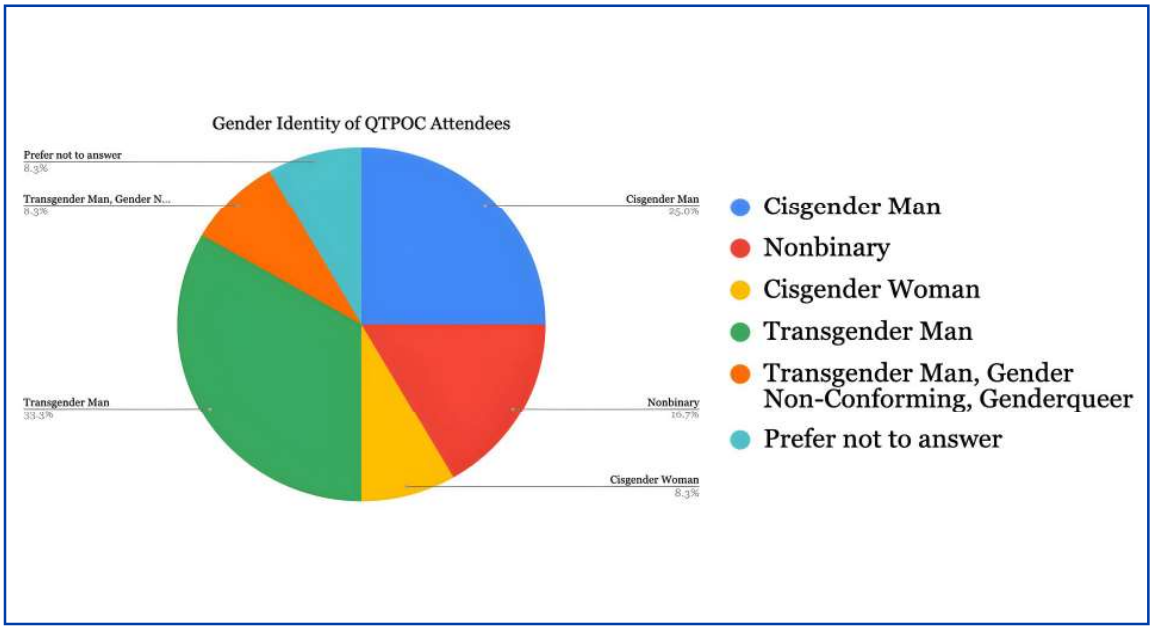

Figure 2c: Gender Identity of QTPOC Attendees

A pie-chart graph of the different gender identities of QTPOC attendees at the eight events.

Of the other categories, there is an even split of 8.3% each of "Queer", "Asexual, Queer", "Pansexual", "Gay, Queer, Other", "Heterosexual/Straight", and "Bisexual, Queer." Overall, 33.2% of QTPoC at QTPOC events identified as being queer solely or in addition to other sexual orientations. Figure 2c depicts the gender identity of QTPoC attendees across eight QTPOC events. 33.3% of attendees self-identified as transgender men, followed by 25% as cisgender men, 16.7% as nonbinary, and 8.3% as cisgender women. The data about race/gender/sexuality shines a light on how, despite all individuals indicating that they identify as QTPoC, they still had different race, gender, and sexuality labels that they identified with. All the identities are self-volunteered information, and participants could select multiple identities or provide their own, if not listed. Additionally, they had the option of "Prefer Not to Answer," respecting their autonomy in disclosing personal information. The spread of answers collected when surveying participants about different parts of the identities indicates that QTPOC events were an inclusive environment, housing a diverse group of QTPoC that were able to benefit from the opportunity to connect with peers to share lived experiences while simultaneously receiving food. Our "Anecdotes" section provides qualitative evidence that suggests the provision and presence of food at QTPOC events was the vehicle for QTPoC to attend.

Figure 3 displays results from an identical question on the pre-and post-event surveys: "What are the names of resources at UCR that can help with food insecurity?" In the pre-event survey data, 77.7% of respondents accurately identified Basic Needs, CalFresh, and the R'Pantry. In the post-event survey data, 89.4% of respondents accurately identified Basic Needs, CalFresh, and the R'Pantry as resources at UCR that can help with food insecurity. This was an 11.7% increase in accuracy from the pre-survey. This increase in knowledge occurred over the course of QTPOC events, therefore it suggests that QTPOC events were responsible for this knowledge boost.

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

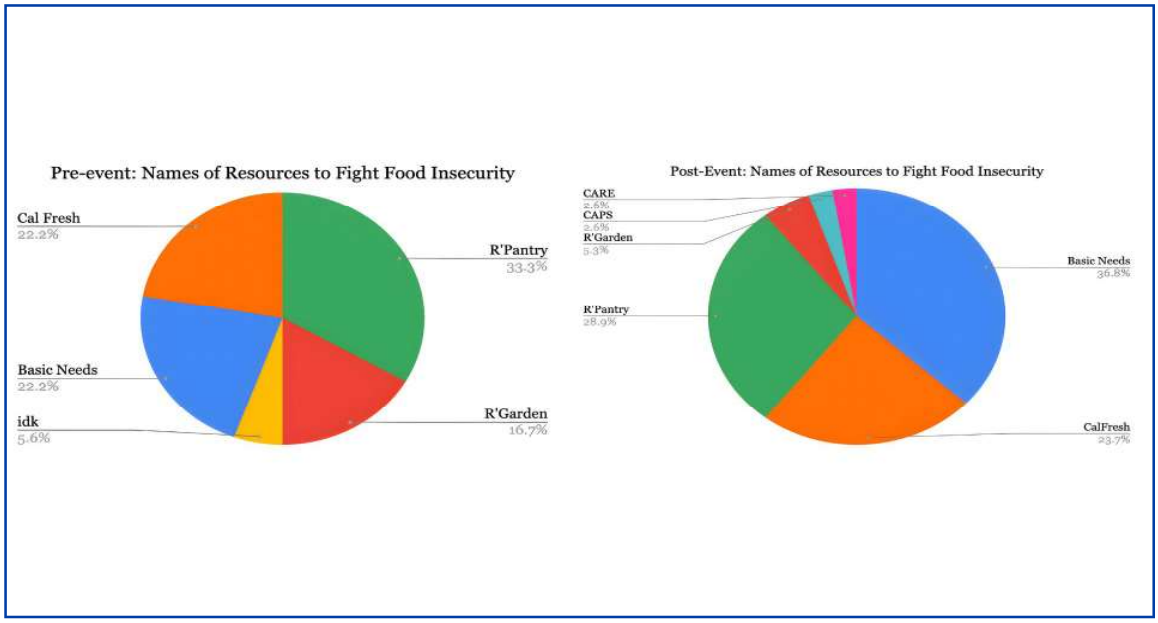

Figure 3: Pre- and Post-event Knowledge of Food Security Resources

A pie-chart graph of the post- and pre-event survey responses of food insecurity resources at UCR. These responses are from QTPOC attendees at the eight events in the Winter Quarter.

Qualitative

We also quoted many student testimonials for various questions such as "What motivated you to come to this QTPOC event?", "Describe how having food at events has contributed to your well-being and belonging.", and "Are there any additional comments or suggestions you would like to share regarding the impact of QTPOC on campus?" from the surveys. Many of the respondents are grateful to have a space that especially offers free meals at the event. We highlighted 3 student testimonials from all eight QTPOC events.

Anecdotes

Pre-Survey Question: "What motivated you to come to this QTPOC event?"

1. "I've been questioning my identity so coming to events like this feels right to me"
2. "What motivated me was the hope to find a supportive group and to be more informed on how I can be a safe space for others"
3. "I wanted to see what the presentation was going to be about, eat some food and actually attend a qtpoc event"

Post-Survey Question: "Describe how having food at events has contributed to your well-being and belonging."

1. "I feel like eating together brings people together, people let their guards down to eat and they take a moment to relax and enjoy the moment they are in"
2. "It made it a space that valued the well-being of each other along with the already welcoming space. It also made it a less awkward space (this being my first time in a QTPOC event)"
3. "I like coming because of the food mostly, I feel better that we're eating together because sometimes I go home without eating because it can be too expensive. I'm glad I stopped by"

The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

Post-Survey Question: "Are there any additional comments or suggestions you would like to share regarding the impact of QTPOC on campus?"

1. "I felt really nervous but I was happily showed that I can become comfortable in this space"
2. "Thanks for making this safe space"
3. "It's very nice to have somewhere to come to"

The anecdotes largely indicate that the primary motivation for QTPoC to attend QTPOC events was because they sought a safe, inclusive space. Participants shared that the provision of food at QTPOC events played an instrumental role in their overall event experience. QTPoC attendees shared that food made the space more welcoming and that it helped bring people together. We provided catered "buffet-style" meals at our events, offering a variety of options including meat, vegan, and vegetarian dishes. This inclusive approach ensured that participants could freely choose the type and quantity of food that suited their preferences. Our food setup featured an appetizer, warm main course, and a selection of fresh fruits.

Figure 4 shows a word bubble of the most used words when describing the pre-survey question: "What motivated you to come to this QTPOC event?". "Friend" had the highest frequency followed by "food." Although guaranteed food was a motivating factor in attending QTPOC events, the possibility of making friends or going to the event with friends was the biggest motivation. We opted not to offer food at two out of the eight Winter Quarter events due to their focus on crafts which encouraged active interaction amongst peers. Many denoted QTPOC as being a "safe space" or a general "space" to be at and marked "supportive group". In addition, the "LGBT Resource Center" was noted as many of our advertisements are displayed by the LGBTRC.

Figure 4: Word-Bubble of Survey Answers

Word bubble of answers for survey question: "What motivated you to come to this QTPOC event?" Responses recorded of all 8 events.

DISCUSSION

Overall, QTPoC students at QTPOC events experienced an improvement in their mental health which was determined via quantitative and qualitative data. QTPoC experienced an average 14.3% increase in reported mental health by the end of QTPOC events compared to before the events. In Figure 4, "friend" is reported by QTPoC participants as being the largest motivator for them attending QTPOC events. The Anecdotes indicate that motivators for QTPoC to attend QTPOC events also included wanting a "supportive space." In our survey, we specifically asked QTPoC how the food at QTPOC events contributed to their mental well-being and belonging, to which they shared that food "made it a less awkward space" and "brought people together." The significance of the Anecdotes and Figure 4 is that they provide insight into the role and significance of food at QTPOC events. While food was appreciated by QTPoC attendees, it was not the sole component that contributed to the improved mental health observed in Figure 1. The food acted as one element contributing to increased mental health outcomes, the other elements being the peer-to-peer interaction and sharing of lived experiences that QTPOC events create as a third space. Additionally, the variance for the pre-survey data (1.130) is larger than the post-survey data (0.834) indicating more

variability in mental health ratings among participants before attending the events compared to after attending them. This showed a stabilization of mental well-being post-event. We predicted that an essential component of QTPOC event turnout was the provision of hot food through our Basic Needs funding, and though it is a main factor with QTPoC participant testimonials to confirm, many had also noted that either making friends or going to an event with friends was the main motivator. From reading the testimonials, we found that food catalyzed QTPoC to attend QTPOC events. Participants shared that "eating together brings people together" and that they felt more at ease knowing that they would not have to worry about food for the night. Through anecdotes and observations, we illustrate how attendees may initially be drawn by the promise of food but ultimately leave with newfound friendships and support networks. This distinction draws attention to the significance of our peer-to-peer approach.

FUTURE DIRECTIONS

As funding for the QTPOC Project from the LGBTRC (UCR) and the Basic Needs Department is expected to change next year, it presents an opportunity for a comparative analysis of the project's effectiveness with varying levels of funding. Our research question revolves around understanding the effectiveness of our method and how it can be replicated by others to create similar "third spaces," which are crucial for intersectional support. We employ various tools in our study, with food acting as a primary motivator alongside the importance of being in a supportive environment and increasing awareness of campus resources.

The food provided at all QTPOC events could not have been possible without funding. Our results demonstrated that food was a key motivator for QTPoC to attend QTPOC events; less funding may have effects on event attendance and data findings. A comparative data analysis will help assess the impact of financial resources on the project's ability to support QTPoC individuals. Also, given that many participants did not complete both pre- and post-surveys, future studies should explore strategies to improve survey completion rates to ensure data cohesiveness.

The QTPOC Project serves to highlight the need for greater funding for LGBTQ+ resources on UC Riverside campus, and we hope our research takeaways will lead to greater funding for organizations–especially those that serve LGBTQ+ populations and other underserved communities. Additionally, we would like to highlight the importance of providing hot meals at events to address the challenges faced by members attending evening meetings, particularly during dinner hours. This initiative aims to ensure that individuals do not have to choose between attending meetings and meeting their basic nutritional needs.

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The Impact of a QTPoC-Serving Student Organization in Combating Food Insecurity and Mental Health Disparities

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