

# VOLUME 14



5) L  
a weak mo.

A)  $1.8 \times 10^{-6}$       B)  $1.3 \times 10^{-2}$

6) Calculate the  $K_{sp}$  for silver sulfate if the solubility of

A)  $2.1 \times 10^{-4}$       B)  $1.2 \times 10^{-3}$

A galvanic cell employs the reaction.

Soaked (min)	Diameter (mm)	Soaked Weight (g)
0.0160		

**explorations**

THE TEXAS A&M UNDERGRADUATE JOURNAL



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**e x p l o r a t i o n s**  
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# THANKS & GIG 'EM!

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Faculty at Texas A&M can mentor undergraduate researchers and encourage them to apply to the board and/or to submit synopses. Faculty and staff can also volunteer to review submissions for *Explorations*. The review process begins each spring semester.

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# FROM OUR FACULTY

Welcome to Volume Fourteen of *Explorations*, documenting the impressive research accomplishments of undergraduate students at Texas A&M University. The work presented here represents the achievements of students from a variety of disciplines who have challenged themselves. As we emerge from a period of unprecedented disruption to our research endeavors it is important to acknowledge the resilience of these students. They began their studies at a time when the greatest benefits of the undergraduate research experience, personal interactions with mentors and team collaborations, were constrained. Yet they persevered, recognizing that students at this university have a remarkable opportunity not just to learn from some of the world's most distinguished researchers, but to participate directly in the process of discovery. Faculty members will tell you that the most gratifying outcome of a mentoring relationship is when the student takes their own path, developing the skills, knowledge, and independence to ask their own questions and design their own approaches, fueled primarily by their curiosity, talent, and desire for discovery. Particularly valued is the ability to communicate the significance of their research. The students who have published in *Explorations* have struck out on that path, not only taking responsibility for their own direction of discovery, but enthusiastically communicating its importance to a broader audience.

We must acknowledge the faculty, graduate students, and staff who have helped these students every step of the way. By documenting the productive interactions of undergraduate students and researchers *Explorations* bears impressive witness to the successful achievement of our educational mission. I also congratulate the *Explorations* Editorial Board, a dedicated group of student leaders who have worked hard to bring together the articles selected for this excellent issue. Having sat through many editorial board meetings during my time as an Associate Director in LAUNCH, I know that their decisions are not taken lightly. I have been consistently impressed by the thoughtfulness of the Board's deliberations when choosing articles which represent the breadth of research at Texas A&M, helping authors refine their writing to communicate more effectively to a general audience, and developing the style of

the final volume.

Upon my retirement I have had the pleasure of reconnecting with a number of my former undergraduate researchers. They now live in far-flung places, from Berkeley to Burlington, and have pursued a diversity of careers, including re-

search, teaching, and industry. Yet they all have retained their sense of wonder at discovery, whether it be at a lab bench, in a high school classroom, at a biotech company, or in a backyard observatory. They unanimously express their conviction that undergraduate research was a highlight of their Texas A&M career, providing them with unique knowledge, earned self-confidence, and lifelong friendships. I will greatly miss the new faces that appeared in my laboratory each year, but I retire knowing that the undergraduate research enterprise remains robust at Texas A&M. There is still much to accomplish: to welcome more first generation students and those from underrepresented groups to the exciting world of research discovery, to pursue more sources of support to reward these students for their initiative, and to engage more students in international research experiences such as those offered through the MSC Jordan Institute for International Awareness. To these ends I commend the staff of the LAUNCH office, who oversee the production of *Explorations*, for their continuing efforts towards these goals. The research described in this volume should inspire future students to seek out the support of the many on this campus who will assist them in attaining similar transformative educational experiences.



A handwritten signature in black ink, which appears to be 'Duncan Mackenzie', written over the bottom right corner of the portrait image.

**DR. DUNCAN MACKENZIE**

**PROFESSOR EMERITUS  
BIOLOGY, TEXAS A&M UNIVERSITY**

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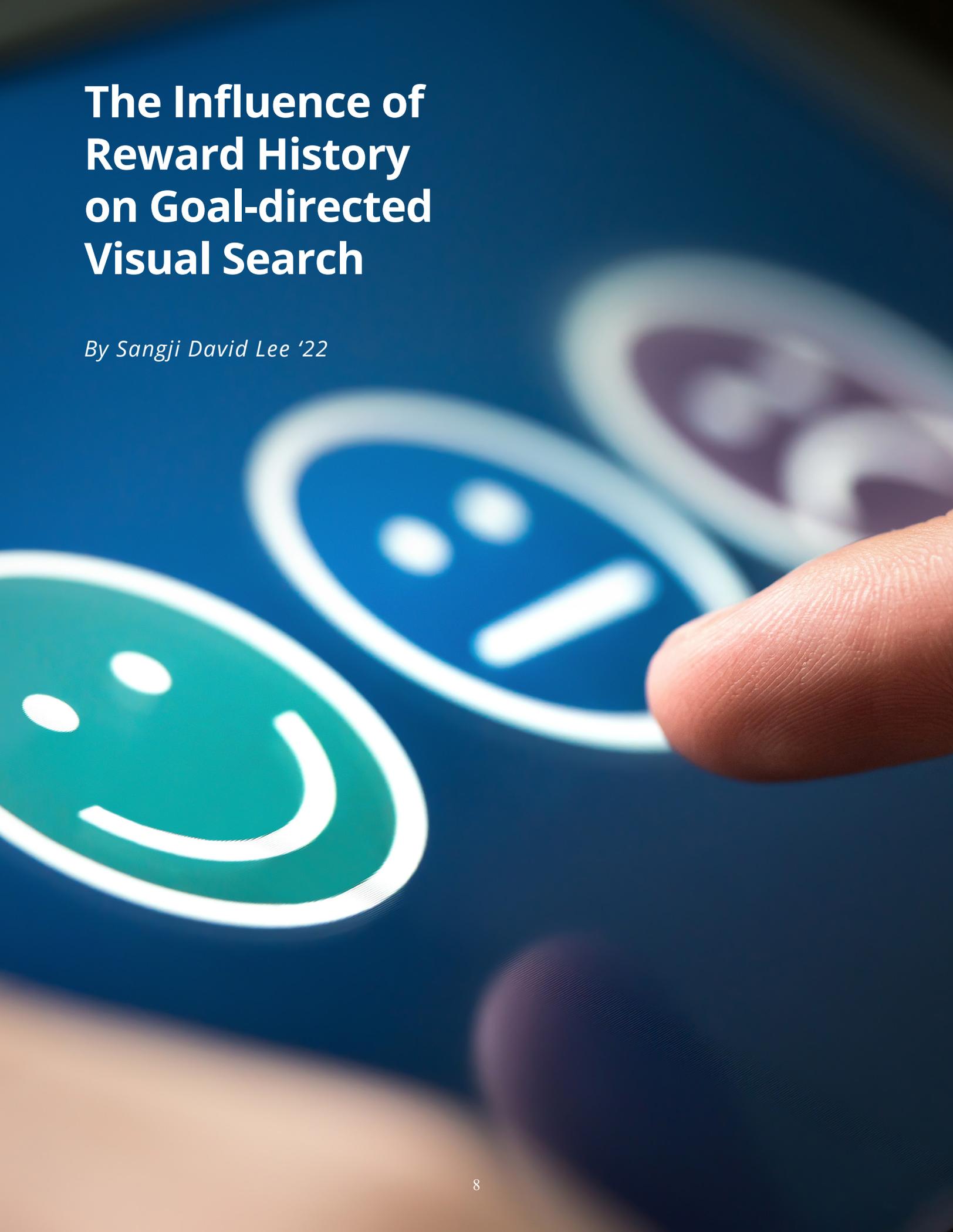
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# The Influence of Reward History on Goal-directed Visual Search

*By Sangji David Lee '22*



## INTRODUCTION

Daily, people encounter an overwhelming amount of visual information, but our perceptual system can only process a relatively small amount of information at any moment. How do our brains manage this limitation? Attention mechanisms determine which objects or locations in a given moment are represented in the brain.<sup>1</sup> By prioritizing items that share visual features (color, shape, etc.) with what you are looking for, attention allows you to find a particular object in your room, like car keys among the pile of things on your desk. To strategically select an item, the attention system compares what you see to a *search or target template*—a mental representation of what you are looking for—and selectively processes objects that sufficiently match the template.<sup>2</sup>

Beyond strategic attentional control, what we pay attention to is influenced by two other factors: physical characteristics of objects (how bright and colorful they are)<sup>3</sup> and learned associations between objects and outcomes (whether they tend to predict good or bad outcomes, like a tasty treat predicts the pleasure of eating it).<sup>4</sup> This study investigates the influence of reward learning on attention. Reward-associated stimuli can become attention-grabbing, which remains the case even when such stimuli no longer predict reward nor are relevant to the current task.<sup>5</sup> The term *value-driven* summarizes this biasing effect of reward on our attention,<sup>6</sup> which is readily seen in everyday life in the context of addiction<sup>7,8</sup> or favor toward items generally associated with reward (e.g., why tasty food on the table is difficult to ignore even when dieting). The prospect of reward can also modulate the goal-directed control of attention by enhancing our focus.<sup>9,10</sup>

Most prior research on attention, including the influence of reward, measures how people process information under conditions in which participants follow explicit instructions concerning a specific stimulus. The question then becomes whether reward makes a certain object more distracting (e.g. whether people are better at finding something when they are rewarded for doing so quickly and accurately). However, real-life visual search does not provide any instruction concerning

## THIS STUDY INVESTIGATES THE INFLUENCE OF REWARD LEARNING ON ATTENTION.

what to find or how to find it. Lack of instruction requires our brain to consider different potential target templates and settle on one to use when searching. In a dynamic environment, the template we use changes to meet current environmental demands. We know reward history can bias attention to specific reward-associated stimuli, but can reward also bias how people choose to strategically search through a display? Given the role reward's influence on attention plays in addiction,<sup>11,12</sup> the answer to this question has implications for our understanding of how drug reward might influence the way in which people direct their attention in the future.

## EXPERIMENT ONE

For Experiment One, we used a paradigm called the “Adaptive Choice Visual Search” (ACVS) task.<sup>13,14</sup> ACVS manipulates the color distribution between red, blue, and green boxes to create a dynamic search environment. In each trial, one red and one blue target were hidden among red, blue, and green boxes. In this study, red and blue are referred to as task-relevant colors and one color is less abundant than the other in each trial. Participants only needed to report one target and could search for either the red or blue target. Searching through stimuli rendered in the less abundant color is easier, or more “optimal” strategy-wise, since there are fewer items to search through. To examine the effect of reward on the choice of how to search, participants went through an initial training phase in which only one of the two task-relevant colors was presented on each trial (red or blue among green). Participants received monetary reward when they found a target in one but not the other color. In the following test phase, both red and blue boxes were present in the same trial, and rewards were no longer available. Each participant

encountered three types of trials:

1. the previously rewarded color was the optimal color to search through,
2. the previously rewarded color was non-optimal to search through, or
3. neither color was optimal to search through (equal number of red and blue items).

We were interested in whether participants would choose to search through the previously rewarded color and how the selected strategy interacts with how optimal it would be to do so.

## Methods

Thirty-five participants (25 female) between the ages of 18 and 35 (mean = 20.3 years) were recruited from the Texas A&M University community. All participants reported normal or corrected-to-normal visual acuity and normal color vision. Participants were compensated with their earnings in the task.

The experiment was run on a computer in a dimly lit testing room, and participants indicated their responses using a button box (like a gamepad). Each visual search array was composed of 54 colored squares arranged in three concentric rings around the center of the screen. The inner, middle, and outer rings consisted of 12, 18, and 24 boxes, respectively. Each box in each ring was positioned equidistant from each other and contained a digit between two and nine.

Following consent, participants completed

**WE WERE INTERESTED  
IN WHETHER  
PARTICIPANTS  
WOULD CHOOSE TO  
SEARCH THROUGH THE  
PREVIOUSLY REWARDED  
COLOR...**

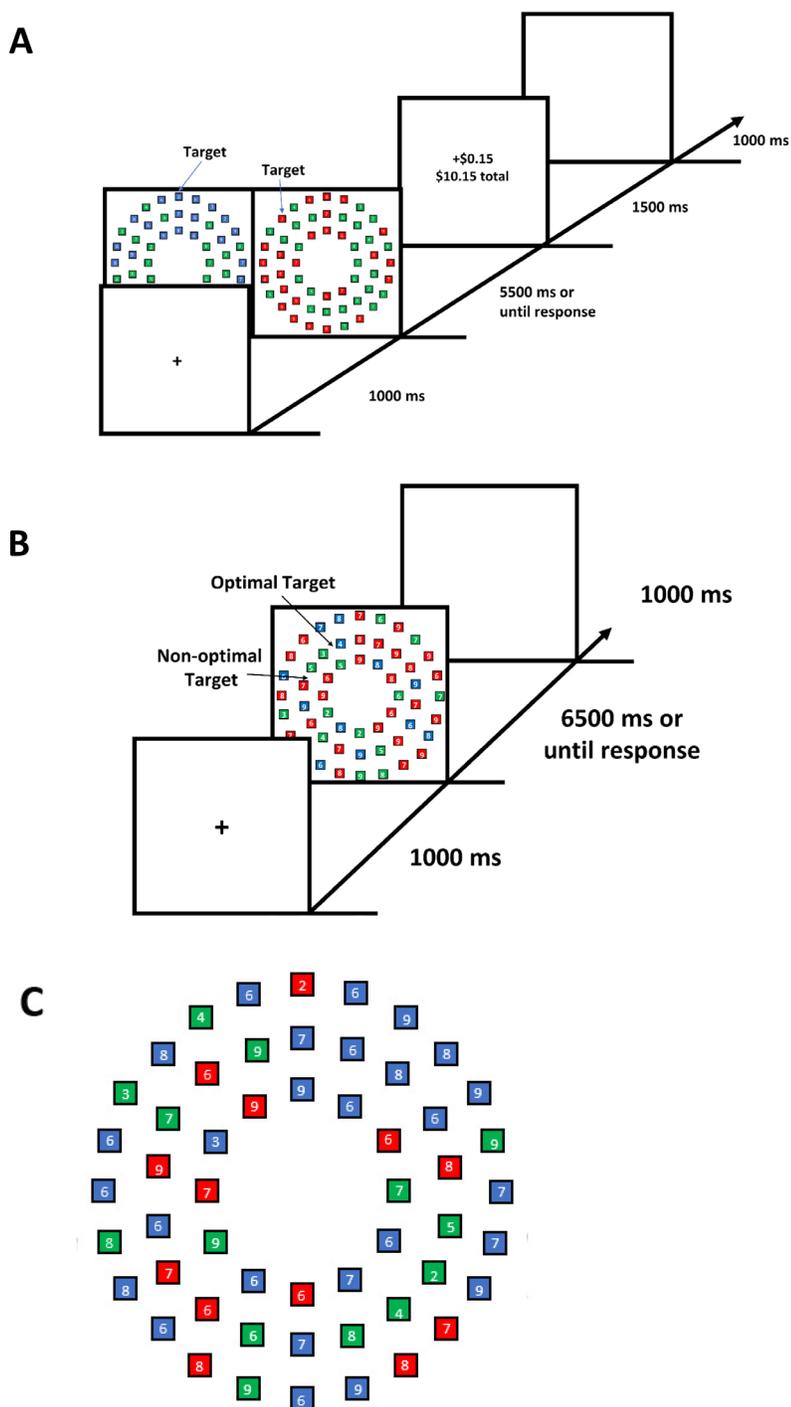
three runs of the training phase followed by three runs of the test phase. Before each phase, participants practiced the task. Importantly, no instructions were given concerning how to search and participants were free to follow the search strategy of their choice.

## Training Phase

The training phase consisted of a fixation display (1,000 milliseconds), search array (5,500 milliseconds or until response), feedback display (1,500 milliseconds), and a blank inter-trial-interval (1,000 milliseconds) (**Figure 1A**). During the training phase, there were two trial types for the search array:

1. red and green color squares, and
2. blue and green color squares.

Participants were instructed to search for a target square: a red or blue color square containing a digit between two and five. An equal number of target- and green-color squares were presented on each trial. All red or blue squares besides the target square contained a digit from six to nine. Green-colored squares were neutral to the task and contained digits between two to nine to prevent participants from searching for numerical digits without respect to color. All digits inside non-target squares were assigned randomly using the aforementioned constraints. One of the two target colors was associated with monetary reward and which color was used as the reward-associated color alternated across participants. Upon identifying a target in the rewarded color, participants were shown “+\$0.15” and their accumulated total earnings. In contrast, upon identifying a target in the unrewarded color, participants were shown “+0.00” and their accumulated total earnings. If participants responded with a number other than the target number, they were presented with the word “Missed” with their accumulated total earnings. If participants did not make a manual response within the 5,500 milliseconds time limit, they were presented with the words “Too Slow” and their accumulated total earnings. For each participant, the training phase consisted of 60 trials (30 trials of each trial type, randomly distributed), and participants took a short break between every three trials.



**Figure 1.** Sequence of trial events for the (A) the training phase, (B) the test phase of Experiment One and (C) search display for the test phase.

## Test Phase

The test phase consisted of a fixation display (1,000 milliseconds), search array (6,500 milliseconds or until response), and a blank inter-trial interval (1,000

milliseconds) (Figure 1B). There were three trial types in the visual search array:

1. previously reward-associated color optimal (trial displayed 13 reward-associated color boxes, 27 non-reward-associated color boxes, and 14 green color boxes),
2. previously reward-associated color non-optimal (trial displayed 27 reward-associated color boxes, 13 non-reward-associated color boxes, and 14 green color boxes), and
3. neutral trial (trial displayed 18 reward-associated color boxes, 18 non-reward-associated color boxes, and 18 green color boxes).

Here, optimality is defined as “optimal for maximizing task performance” in that it would be faster to search through the color with a smaller set-size. Participants were informed that one red and one blue target would be present in each trial (red or blue color square containing a digit from two to five) and they only needed to report one of these targets. Non-target squares were assigned numbers in the same manner as in the training phase. Feedback was inserted after the search array if participants responded incorrectly or failed to respond before the time limit.

For each participant, each testing phase consisted of three blocks which required search tasks 90 times each (30 trials of each trial type, randomly distributed). A short break was given between each block.

## Data Analysis

We excluded data from three participants due to low accuracy in the task (< 3 SD of the group mean)

and one participant withdrew before completing the task. Thus, 31 data sets were fully analyzed.

## Results and Discussion

### Training Phase

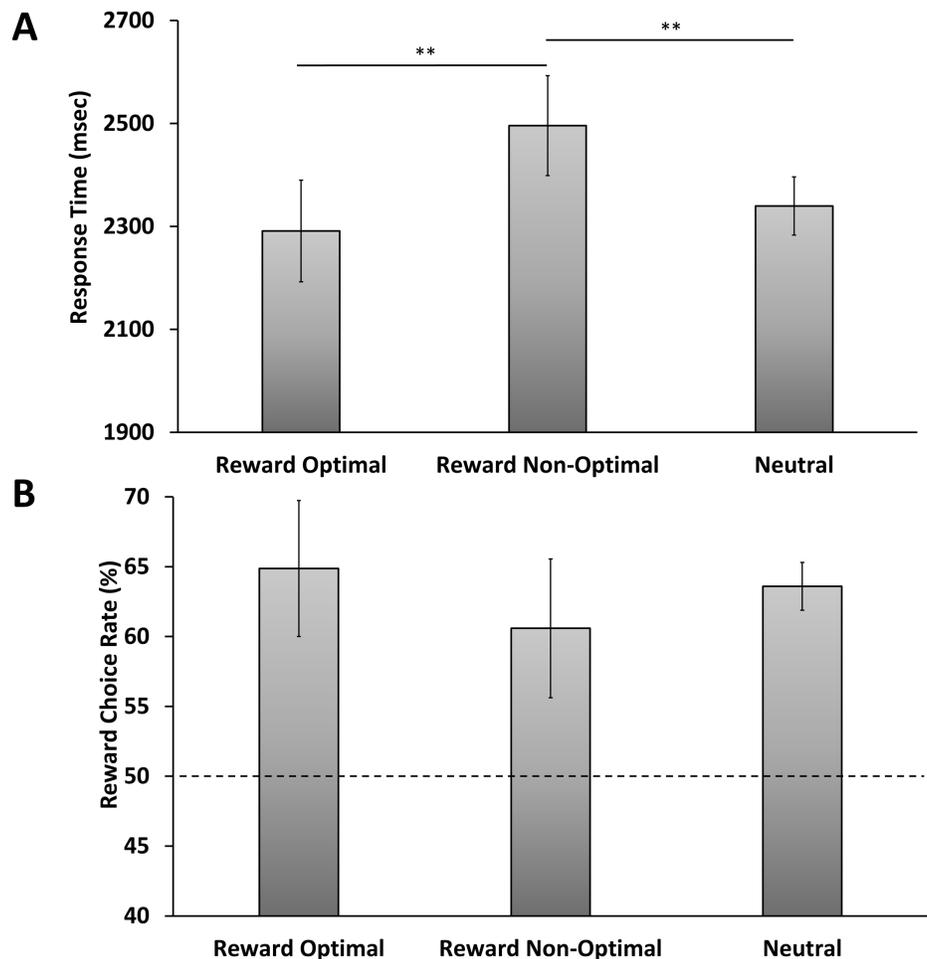
Neither response time nor accuracy differed as a function of the reward given by the target color,  $t(30) = 0.47, p = .641$  (reward: mean = 2,809 milliseconds, no-reward: means = 2,829 milliseconds), and  $t(30) = 0.00, p > .999$  (reward: mean = 93.9%, no-reward: M = 93.9%), respectively.

### Test Phase

We conducted a one-way repeated-measures analysis of variance (ANOVA) over response time with trial type as a factor (reward optimal, reward non-optimal, neutral), which revealed a significant difference across trial types,  $F(2,60) = 6.41, p = .003, \eta_p^2 = .176$  (see **Figure 2A**). The  $F(\#, \#)$  indicates the score of the significance of the one-way repeated-measures analysis of variance and  $p$  measures the validity of the  $F(\#, \#)$  score.  $H_p^2$  measures the variance within the dependent measurement. Post-hoc pairwise comparisons identified participants were faster in trials when the reward-associated color was optimal compared to non-optimal,  $t(30) = 2.73, p = .010, d_z = 0.49$ , slower on trials when the reward-associated color was non-optimal compared to neutral trials,  $t(30) = 3.14, p = .004, d_z = 0.56$ , and no differences comparing trials when the

reward-associated color was optimal and neutral,  $t(30) = 0.95, p = .352$ .  $t(\#)$  indicates the score of the significance of the t-test and  $p$  measures the validity of the  $t(\#)$ .  $D_z$  shows the variance in the mean.

To assess whether reward history influenced choice behavior, we compared the percentage of targets found in the previously reward-associated color to chance (50%, which would reflect unbiased search). Participants were significantly biased to report targets rendered in the previously reward-associated color,  $t(30) = 2.53, p = .017, d_z = 0.45$ . In contrast, excluding neutral trials in which there was no optimal strategy, participants were not more likely to select the optimal-color target than chance,  $t(30) = 1.10, p = .278$ .



**Figure 2.** Behavioral results in the test phase. **(A)** Response time and **(B)** the rate of choosing a previously reward-associated color target when it was the optimal color to search through, the non-optimal color, or when there was no optimal color in a neutral condition. Error bars depict within-subjects confidence intervals calculated using the Cousineau method with a Morey correction. \*\*Statistically significant,  $p < 0.01$ .

We then ran an ANOVA over the percentage of targets found in the previously reward-associated color over the three trial types and found no significant difference,  $F(2,60) = 1.18, p = .316$  (**Figure 2B**). This finding means participants were overall biased to report a target in the previously reward-associated color regardless of how optimal this strategy was with respect to the distribution of color stimuli.

In addition, we found significant differences in accuracy across trial type,  $F(2,60) = 4.63, p = 0.013, \eta^2_p = 0.134$ . Post-hoc pairwise comparisons identified that participants were more accurate on neutral trials compared to both reward-optimal,  $t(30) = 2.49, p = .018, d_z = 0.45$  (neutral: mean = 96.9% optimal: mean = 95.2%), and reward non-optimal trials,  $t(30) = 3.65, p < .001, d_z = 0.66$  (non-optimal: M = 95.1%). Reward-optimal and reward non-optimal trials did not differ from each other,  $t(30) = 0.23, p = 0.822$ . These differences may be related to the fact that the varying distribution of color stimuli made it so neutral trials had a slightly higher number of green stimuli (which could not be targets).

Overall, participants were more likely to report a target rendered in the previously rewarded color compared to a target in the previously unrewarded color in the test phase, suggesting that they preferentially searched through stimuli of the previously rewarded color. The bias was evident even when the previously rewarded color was the more abundant color in the search display, resulting in a performance cost as reflected in slower response time. Reward history influenced the strategy participants adopted to find a target in a situation in which they were free to choose how to conduct their search.

## EXPERIMENT TWO

It remains unclear how “optimal” search would have been in the absence of reward history in Experiment One, and whether the reward-biased strategy that participants adopted caused them to abandon an otherwise optimal strategy when the previously reward-associated color was more abundant. Although preferentially searching for the previously reward-as-

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TO CONDUCT THEIR  
SEARCH.**

sociated color came at a cost in response time when it was the more abundant color, participants may have otherwise tended toward strategies that do not prioritize the less abundant color, such as searching serially through all red and blue stimuli until a target is found. In prior implementations of the ACVS task, there are typically no neutral trials, and there is always a less abundant task-relevant color on every trial.<sup>15,16,17</sup> The neutral trials in Experiment One provided an opportunity to assess any reward history-related bias in the absence of a more optimal strategy, and it is possible that the presence of such trials reduced the overall likelihood that participants would realize or come to favor a strategy of preferentially searching through the less abundant color. To characterize performance in the absence of differential reward history as a baseline, we had a separate group of participants complete the test phase from Experiment One without any prior training phase. We were interested in the extent to which participants would favor reporting a target in the less abundant color in trials in which the distribution of red to blue stimuli was imbalanced.

### Methods

Thirty-five new participants (21 female), between the ages of 18 and 35 (mean = 18.3 years), were recruited from the Texas A&M University community. All participants reported normal or corrected-to-normal visual acuity and normal color vision. Participants were

compensated with course credit.

Experimental set-up was identical to Experiment One, except participants only performed the test phase of Experiment One.

## Data Analysis

We excluded data from two participants due to low accuracy in the task ( $< 3$  standard deviation of the group mean) and one participant withdrew before completing the task. Thus, 32 data sets were fully analyzed. Trials were broken down by whether there were more red than blue stimuli or vice versa (imbalanced) or an equal number of red and blue stimuli (neutral). For imbalanced trials, the percentage of optimal targets reported (percentage of targets reported in the less abundant color) was computed and compared against unbiased choice (50%).

## Results and Discussion

Neither response time nor accuracy differed between imbalanced and neutral trials,  $t(31) = 1.51$ ,  $p = 0.142$  (Imbalanced:  $M = 2,762$  milliseconds, Neutral:  $M = 2,714$  milliseconds), and  $t(31) = -1.67$ ,  $p = 0.105$  (Imbalanced:  $M = 93.2\%$ , Neutral:  $M = 94.0\%$ ), respectively. For the imbalanced trials, the percentage of optimal targets reported was not significantly different from chance,  $t(31) = -0.30$ ,  $p = 0.770$  ( $M = 49.4\%$ ). Overall, we found evidence that, without reward history, participants did not exhibit a tendency towards the optimal search strategy in our task, and by extension, reward history did not cause people to abandon an optimal strategy in Experiment One.

## OVERALL DISCUSSION AND CONCLUSIONS

We discovered reward history biased how participants chose to search. They prioritized the previously rewarded color in their search overall, even when it was disadvantageous to do so with respect to task performance and there were no longer any reward incentives in place. This bias toward the previously rewarded color was shown in the rewarded color choice rate.

Our findings expand understanding of how

reward history modulates the control of attention, extending its influence from biasing attention to specific stimuli<sup>18,19,20,21</sup> to influencing the strategic control of attention. More specifically, reward history biases the choice of which target template to use in different situations. From Experiment Two, we learned the bias toward the strategy that has been rewarded in the past does not necessarily occur at the expense of an otherwise optimal strategy. Rather, at least in our task, reward history biases how people choose to search when they otherwise lack a strong inclination based on the task and hand.

Understanding the effect of reward in goal-directed attentional control can provide a new approach for viewing addiction. Not only might a pattern of drug abuse result in biased attention to drug-related stimuli,<sup>22,23</sup> but it could also influence the method or strategy of visual search in the daily life of drug-dependent individuals.

## ACKNOWLEDGEMENTS

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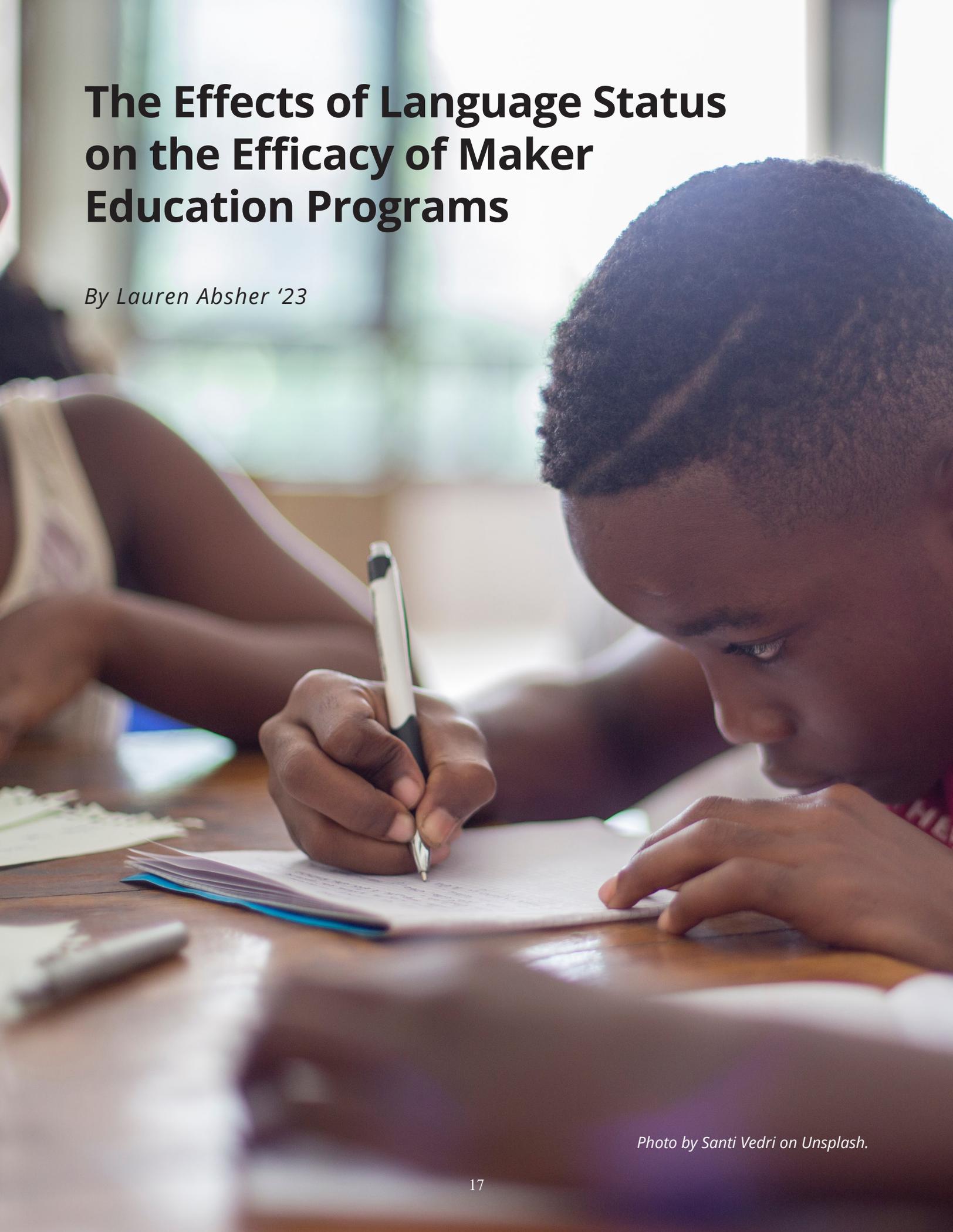


**SANGJI DAVID LEE '22**

Sangji (David) Lee '22 is a biomedical science major from Plano, TX. Sangji went to Plano West Senior Highschool and, through research, plans to enlighten society with novel findings. Sangji is planning to join the public health field as an epidemiologist.

# The Effects of Language Status on the Efficacy of Maker Education Programs

*By Lauren Absher '23*



*Photo by Santi Vedri on Unsplash.*

## INTRODUCTION

There is a growing trend in education called the “Maker Movement.”<sup>1</sup> It aims to enhance students’ science, technology, engineering, and mathematics (STEM) skills, and other important skills, including problem-solving and creativity, by combining technology with education. Maker programs often include experiments, creative projects, and presentations that require students to utilize technology like circuits, 3-D printers, and coding.<sup>2</sup> These programs intend to encourage students to learn about technology, improve their problem-solving skills, and increase their interest in STEM subjects and careers.

One area of particular importance is underrepresented students, as people of color and non-native English speakers are underrepresented in STEM careers.<sup>3</sup> The hope of many maker programs is to encourage historically disadvantaged students to develop an interest in STEM subjects and careers where they are currently underrepresented.<sup>4</sup> It is important to provide these opportunities for underrepresented groups because they have historically been excluded from STEM fields. Previous research has found that students from diverse urban areas responded positively to maker programs, showing an increase in career aspirations and technical skills.<sup>5</sup>

Many maker programs were created as afterschool programs at libraries or other private institutions. However, this makes maker programs less accessible to students from families who do not have the means to attend these programs. To increase accessibility to these programs, some research has aimed to incorporate maker programs into standard public-school education. These programs have both reported a positive impact on students overall.<sup>6,7</sup>

Recent studies have found evidence of maker programs’ effectiveness;<sup>8</sup> however, few have studied maker programs’ impact on multilingual students, who also tend to be underrepresented in STEM careers. This research studies the effect of language status as a moderator for students’ responses to a STEM mentor program. If language status has a negative correlation with

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how multilingual students respond to the mentor program, it could necessitate a change in mentor programs to make them more accessible for underserved students. This study builds upon previous research conducted at Texas A&M University that studied the effects of maker programs on elementary school children’s self-identity and future career aspirations.<sup>9,10</sup> This study consists of the same program as the previous research, except it focuses specifically on the language status of students. We hypothesize that fluent English-speaking students will show a higher increase in STEM self-efficacy and STEM career interest than students with low English proficiency (LEP) in response to maker programs implemented in their schools.

## BACKGROUND

### *Self-Efficacy*

In psychology, self-efficacy is defined as people’s beliefs in their ability to perform a task.<sup>11</sup> A strong STEM self-efficacy indicates that an individual believes they are highly capable of succeeding in STEM related tasks and learning STEM concepts. Research shows that there is a positive effect of self-efficacy on students’ academic achievement and aspirations.<sup>12</sup>

### *Career Interest*

STEM career interest is defined as a student’s desire to pursue STEM-related careers and their beliefs about what career they will have in the future. Studies show that children’s experiences and beliefs affect

what careers they pursue later in life.<sup>13</sup> Therefore, it is important to develop and cultivate interest in STEM subjects in children while they are young so their interests continue later in life, increasing the likelihood they will pursue STEM careers.

## METHODS

### *Making*

The maker program developed for this experiment consisted of three weeks of making activities. Each week focused on a different science unit. The activities were designed to build on the topics that students encountered in their sciences classes. The lessons were also designed to build upon each other, culminating in the students completing one project each week. The later weeks also relied on skills learned in earlier weeks of the program. For example, one week, students learned about circuits and electricity, and then in later weeks they had to build circuits to complete the project for that week.

The lessons were created by a team of professors and educators with knowledge in science, engineering, and educational development. The maker projects differed from standard classroom lessons and experiments because the maker projects required students to use technology to solve problems.

### *Participants*

A total of 212 participants were involved in this study. The participants consisted of 127 (59.9%) third-grade students and 85 (40.1%) fourth-grade students ranging from ages eight to twelve. Of the third-grade students, 48 (37.8%) spoke English fluently and 79 (62.2%) had low English proficiency. Of the fourth-grade students, 36 (42.4%) spoke English fluently and 49 (57.6%) had low English proficiency. The number of students in the statistical analysis varies because some of the data were incomplete. The discrepancy between the data sets is due to some students filling out sections of the surveys incorrectly, requiring those sections be omitted from the analysis. Additionally, a few students' entire pre- or post-surveys were missing. This was likely caused by students moving out of or into the school

district in the middle of the semester or their surveys being misplaced.

The experiment was conducted at a local elementary school in East Texas. The school chose five classes to participate in the experiment.

### *Design*

This experiment took place over the course of one semester. The intervention was split up into three one-week long sections. The school's curriculum was set up so they had three academic periods lasting six weeks per semester. The interventions occurred once per six weeks and corresponded with the topic that students were learning at that time. The students were given two identical surveys, one at the beginning and one at the end of the semester.

### *Measures*

The surveys consisted of two portions. In the first portion, students wrote jobs they thought someone who was good at either math or science would have. Students then rated how much they would like to have that job on a 1 to 5 Likert-type scale, with 1 corresponding to "Not at all" and 5 to "A lot." The second part of the surveys utilized a "structured alternative format" adapted from The Self-Perception Profile for Children (SPPC).<sup>14</sup> This questionnaire has been validated to measure children's self-esteem.<sup>15</sup>

The structured alternative format section gave two statements that are opposites (e.g., "I like science" and "I don't like science"). Then the students rated their agreement with the statements on a Likert scale from 1 to 4, with 1 corresponding to "Really not true for me" for the first statement, and 4 corresponding to "Really true for me". When calculating the variables, statements indicating high self-efficacy and self-esteem were scored as being the highest numbers and the statements indicating low self-efficacy and self-esteem were scored as the lowest numbers.

### *STEM Self-Efficacy*

The students' STEM self-efficacy was measured using the SPPC questionnaire described earlier. The students chose from statements like "Other kids

worry about whether they can do their assigned science work” and “Some kids feel like they are good at science.” A student with high STEM self-efficacy would theoretically choose “Really true for me”, or 4 on the Likert scale, in response to the second statement.

## STEM Career Interest

STEM career interest was measured using two variables: the SPPC questionnaire, which in later analysis is referred to as *Job-Good*, and Likert scale questions that asked how much the students would like to have the STEM job that they identified, which is referred to as *JobLike*. For the SPPC questionnaire items, the students chose from statements including “I probably won’t have a job that uses math” and “Someday I might have a job that uses math.” A student with high STEM career interest would theoretically choose “Really true for me” to the second statement, “Someday I might have a job that uses math,” which would correspond to choosing 4 on the Likert scale.

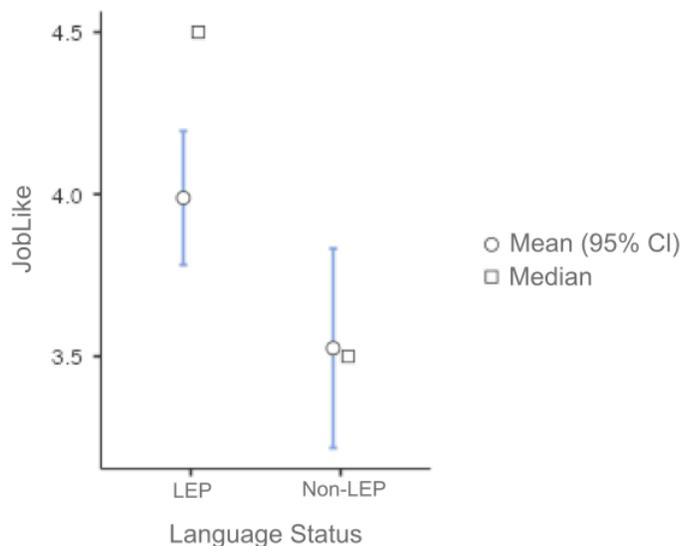
## RESULTS

To determine whether the maker programs influenced the students, three statistical tests were conducted for each variable: 1) Chronbach’s alpha, a reliability analysis, was used to determine how closely related the survey items are as a group, with a score of one being the strongest reliability and zero being weakest, to ascertain how reliable the measure is; 2) an independent samples t-test was used to determine if there was a difference in the LEP and non-LEP students’ final scores; and 3) a mixed measures ANOVA was used to determine if there was any interaction between the LEP and non-LEP students’ pre-and post-test scores.

### STEM Self-Efficacy

The reliability analysis for *STEM Self-Efficacy* found that the questions on the survey used to calculate the variable *STEM Self-Efficacy* were moderately correlated ( $\alpha = 0.656$ ), meaning the variable *STEM Self-Efficacy* is somewhat reliable.

### Composite-JobLike



**Figure 1.** The effect of language status on variable *JobLike*. The bars represent a 95% confidence interval.

The results for the *STEM Self-Efficacy* t-test and mixed measures ANOVA were not significant.

### STEM Career Interest

The reliability analysis for *JobLike* was low ( $\alpha = 0.482$ ), meaning the questions used to calculate were not well correlated, so this variable might be unreliable.

The results of the t-test for the two-level predictor variable, *Language Status*, predicting the *STEM Career Interest* variable *JobLike* are shown in **Figure 1**.

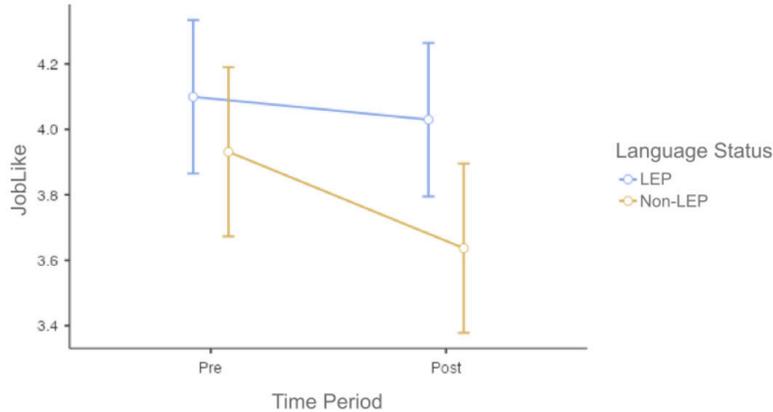
The LEP students scored higher ( $M = 3.99$ ,  $SD = 0.988$ ,  $n = 88$ ) on the variable *JobLike* at the end of the program than the non-LEP students ( $M = 3.52$ ,  $SD = 1.216$ ,  $n = 60$ ). This difference was statistically significant,  $t(146) = 2.55$ ,  $p = 0.012$ , 95% CI [0.1043, 0.8230], meaning LEP students reported liking the STEM career that they identified more than the non-LEP students at the end of the program.

The results of the mixed measures ANOVA test on *Language Status* as compared to the *STEM Career Interest* variable *JobLike* are shown in **Figure 2**.

Results indicated a significant main effect for language status, such that LEP students rated higher ( $M$

### Estimated Marginal Means

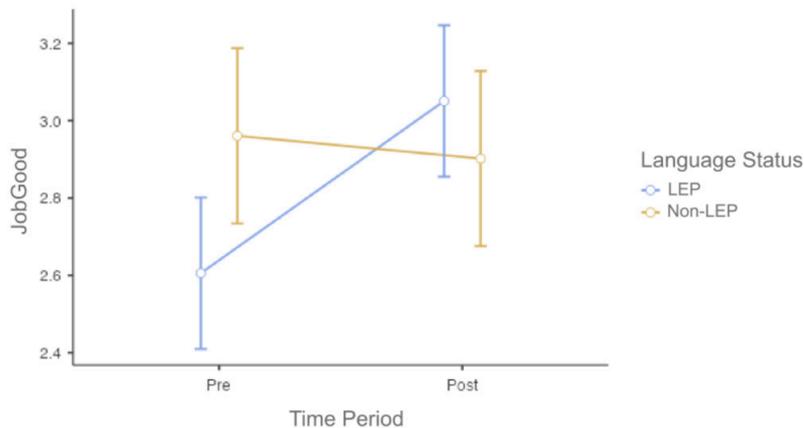
Time Period \* Language Status



**Figure 2.** Repeated measures ANOVA comparing language status and variable JobLike. The bars represent a 95% confidence interval.

### Estimated Marginal Means

Time \* Language Status



**Figure 3.** Repeated measures ANOVA comparing language status and variable JobGood. The bars represent a 95% confidence interval.

= 3.99,  $SD = 0.988$ ,  $n = 88$ ) on the JobLike outcome variable when compared to non-LEP students ( $M = 3.52$ ,  $SD = 1.216$ ,  $n = 60$ ),  $F(1, 140) = 4.51$ ,  $p = 0.036$ ,  $\eta^2 = 0.017$ . This shows that LEP students reported liking the STEM career that they identified more on average than the non-LEP students throughout the program.

The reliability analysis for JobGood showed that the questions used were moderately correlated ( $\alpha = 0.744$ ), meaning the variable is reliable.

The results of the mixed measures ANOVA

test on Language Status predicting the STEM career interest variable JobGood are shown in Figure 3.

Based on the mixed measures ANOVA for the variable JobGood, LEP students started lower ( $M = 2.62$ ,  $SD = 0.832$ ,  $n = 96$ ) than non-LEP students ( $M = 2.83$ ,  $SD = 0.699$ ,  $n = 54$ ). The LEP students' scores increased ( $M = 3.03$ ,  $SD = 0.554$ ,  $n = 71$ ) and surpassed non-LEP students' scores, which decreased slightly ( $M = 2.86$ ,  $SD = 0.810$ ,  $n = 46$ ). The interaction between these variables was significant,  $F(1, 93) = 5.40$ ,  $p = 0.022$ ,  $\eta^2 = 0.028$ . This shows that LEP students' desire to have a job that uses STEM subjects increased throughout the program and surpassed non-LEP students, whose reported interest in careers that use STEM subjects decreased slightly.

## CONCLUSION

### Discussion

This study focused on the effects of a maker program on students' STEM career interest, STEM self-efficacy, and the moderation of said effects by the students' language status. We predicted that low English proficiency would have a negative effect on how much the mentor program affected the students' reported self-efficacy and career interests. The data showed that LEP students had greater increases on their reported STEM career interest and ended with higher scores than the non-LEP students.

Additionally, LEP students ended with scores significantly higher than non-LEP students for the variable JobLike, which measured how much the students would like to have a STEM career. This suggests that

## **A MAJOR ISSUE IN STEM IS THE LACK OF DIVERSITY AND REPRESENTATION OF MINORITY GROUPS IN STEM MAJORS AND CAREERS.**

LEP students were more likely to want to have a STEM career after the intervention. Additionally, there was a significant interaction between the LEP and non-LEP students' scores on the variable *JobGood* which measured how much the students thought they might like to have jobs that use STEM related skills. For this variable, the LEP students started with lower scores and ended with higher scores than the non-LEP students. The significant interaction suggests that STEM intervention through the form of maker programs potentially affects LEP students to a greater degree than non-LEP students.

One possible explanation for these trends is that LEP students may have less experience with maker programs, so when they were exposed to this program, it had a greater impact because the program was a newer experience for the LEP students. Moreover, the differences could be due to the nature of the program, which encourages students to figure out the answers to problems on their own and then create solutions using technology. This might allow LEP students to think in their native language, because less English is needed to perform these tasks than what might be required for activities in a traditional classroom, like essay writing.

Our results have important implications for maker programs in schools. A major issue in STEM is the lack of diversity and representation of minority groups in STEM majors and careers. These results show that maker programs have a greater impact on LEP students' career interests than on non-LEP students meaning that maker programs could be an effective way to increase LEP students' representation in STEM

careers. Additionally, it highlights how important it is to increase LEP students' access to programs like these. Since these programs are more effective at increasing LEP students' STEM career interest than non-LEP students, it would be more cost-effective to ensure that these programs are implemented in areas with larger populations of LEP students, where they would have greater impact. More analysis is needed to clarify the full impacts of maker programs on LEP students and the best ways to implement these programs.

### *Limitations*

There were several limitations to this experiment. First, there was no control group as all students surveyed were in the mentor program. This could result in trends in the general population of students that caused the effects seen.

Second, due to the population studied, most students with low English proficiency spoke Spanish as their native language. This could cause issues when generalizing findings to LEP student populations as a whole because many students with LEP speak languages other than Spanish as their first language. This might impact the effects of the maker program. Additionally, because there were so many students with low English proficiency in this school, there could be a buffering effect due to the social support of other LEP students that might not have been otherwise present if the LEP students were in classes where they were the only non-native English-speaking student.

Third, the surveys were completed using self-report measures. Self-report measures can have reliability issues due to confounds like demand effects. Students might have felt pressured to choose answer choices indicating a positive effect of the program because they felt that the researchers were wanting them to do so.

Finally, the variables had relatively low Chronbach's alpha scores, meaning the scale items were not well correlated with each other, which could cause some variance, preventing the data from being significant. It is possible that if this experiment were repeated with better corresponding variables, the data might show more significant trends.

## ACKNOWLEDGEMENTS

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This project required approval from the Texas A&M University Research Compliance & Biosafety office.

TAMU IRB #: 2019-1132D Approval Date:  
10/30/2019 Expiration Date: 09/12/2022

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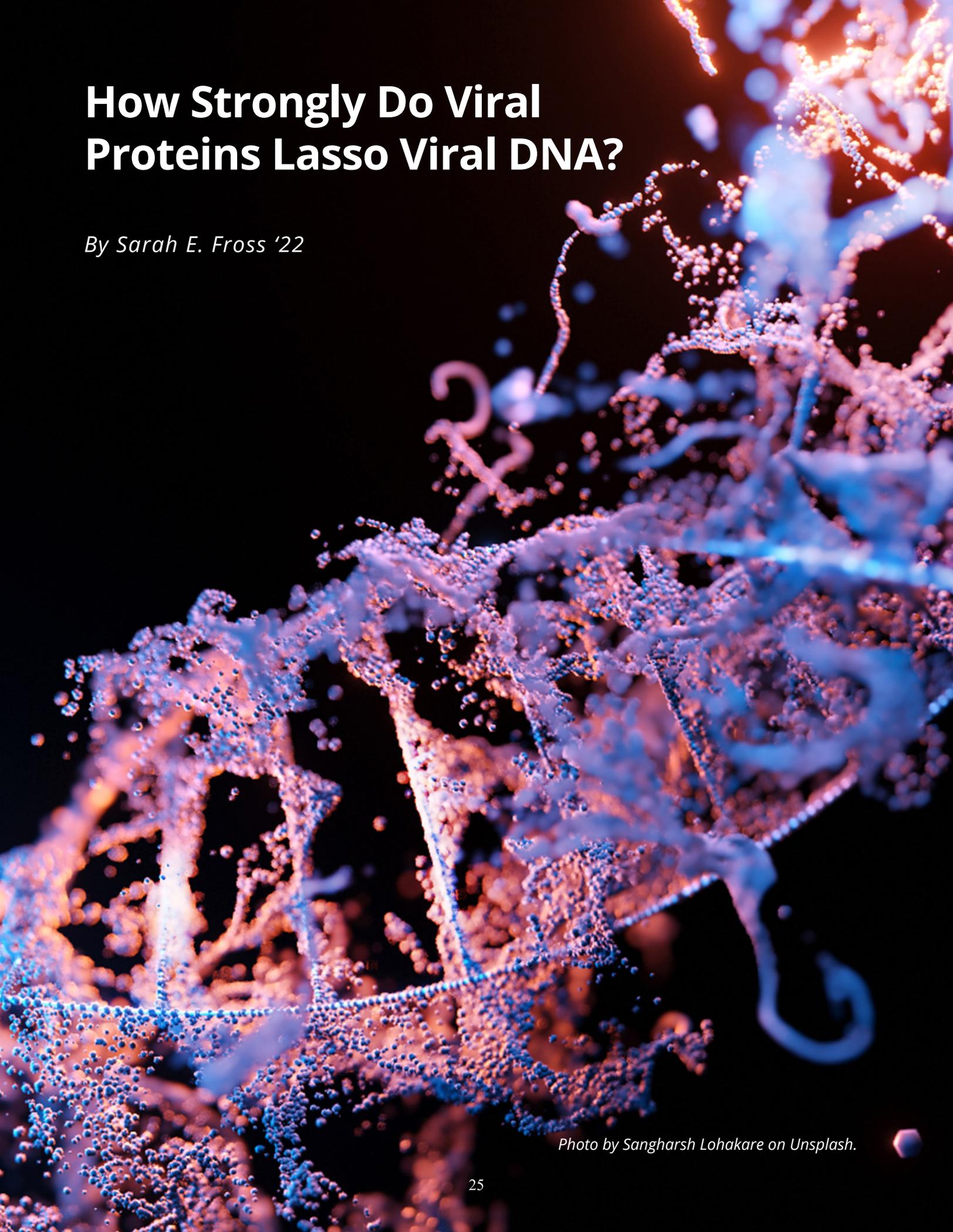


### **LAUREN ABSHER '23**

Lauren Absher '23 is a recent graduate with a psychology major and a neuroscience minor from Plano, Texas. Lauren has been working in the TAMU Existential Psychology Laboratory since the Fall of 2019. She plans to apply to medical schools this year with the ultimate goal of becoming a psychiatrist.

# How Strongly Do Viral Proteins Lasso Viral DNA?

*By Sarah E. Fross '22*



*Photo by Sangharsh Lohakare on Unsplash.*

## BACKGROUND

Viral replication typically involves a protein binding to a specific region of viral DNA (vDNA) to assist in DNA replication and other functions associated with viral infection.<sup>1</sup> The Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) can cause epithelial and lymphatic cancers which are prevalent worldwide.<sup>2</sup> Most often, these viruses are not metabolically active and thus hard to detect during "latency phases."<sup>3</sup> However, the EBV Nuclear Antigen (EBNA1) and KSHV Latency-Associated Nuclear Antigen (LANA) are still active throughout the latency phases. Both EBNA1 and LANA cause cancer by binding to vDNA, increasing cell proliferation and vDNA replication, inhibiting cell-programmed death, and making the infected cell undetectable to the immune system.<sup>4,5</sup>

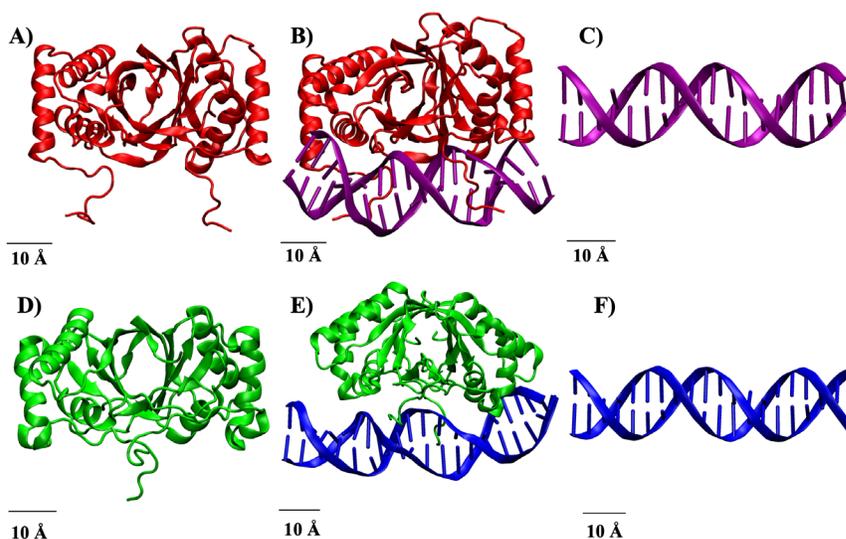
EBNA1 has a role in all activity of EBV's latency phases, making it an appealing target for developing a treatment to combat EBV, as LANA is for KSHV. This study compared the EBNA1 relative binding free energy (RBF) against the LANA RBF obtained through all-atom molecular dynamics (MD) simulations. RBF describes the energy required to create binding interactions, and this study focused on those of viral protein-DNA complexes. With the calculation of the RBF for EBNA1 and LANA, we hoped this could be used for inhibitor development to stop proteins like EBNA1 from binding to vDNAs and provide a preventative treatment for the cancers they cause. Specifically, ligands with RBF values similar to that of either EBNA1 or LANA upon binding to vDNA could be used as protein-inhibitors.<sup>6</sup> Normally, these inhibitors would be discovered through high-throughput screening where up to millions of molecules of varying structure are

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tested to find the most functional drug.<sup>7</sup> By forgoing these trial-and-error binding experiments, binding affinity studies such as MD analyses can streamline drug development to lower material and time costs.<sup>8</sup> To perform these analyses, we studied the thermodynamic and structural properties of EBNA1 and LANA.

## METHODS

MD simulations were performed using the Texas A&M University High Performance Research Computing (TAMU HPRC) and molecular modeling softwares, namely CHARMM and OpenMM (OMM). The viral protein-DNA complexes were obtained from



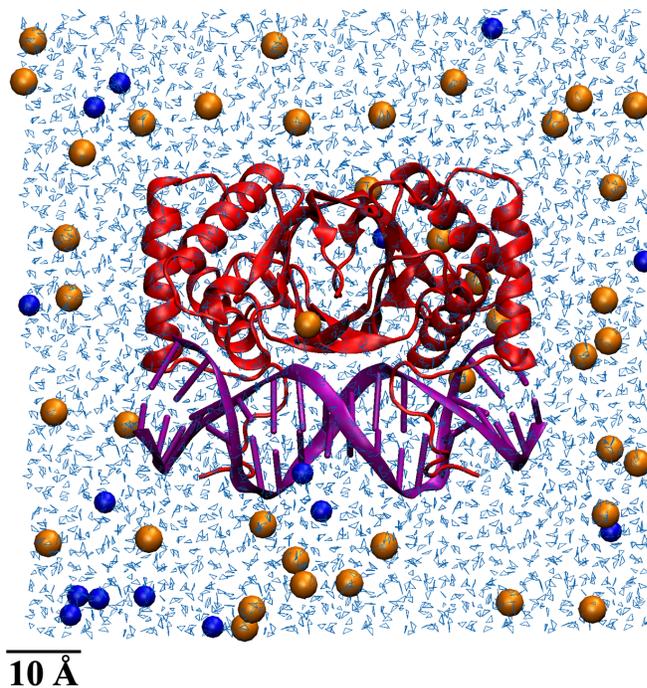
**Figure 1.** Structure of Viral Protein, vDNA, and Protein-vDNA complexes. **A)** EBNA1. **B)** EBNA1-vDNA<sup>EBV</sup> complex. **C)** vDNA<sup>EBV</sup>. **D)** LANA. **E)** LANA-vDNA<sup>KSHV</sup> complex. **F)** vDNA<sup>KSHV</sup>.

the RCSB Protein Database and the respective vDNA strands for EBV and KSHV were constructed using software specific to modeling DNA.<sup>9</sup> The structures were then allowed to reach a more stable, lower energy conformation. The resultant structures are shown in **Figure 1**.

To simulate physiological conditions, water molecules were added to surround the structure in the same fashion as Hwang et al. to allow for the thermal fluctuations that influence the microscopic movement of all other molecular structures.<sup>10</sup> The atomic scale is reported in Angstroms ( $\text{\AA}$ ); 1  $\text{\AA}$  is equal to 0.1 nanometers. Each structure was placed in a cubic box that had faces at least 12  $\text{\AA}$  away from the structure's atoms. The 12  $\text{\AA}$  distance was chosen as the cutoff distance to prevent nonbonded interactions between the molecule and water or another molecule. If the oxygen in a water molecule was within 2.8  $\text{\AA}$  of a structure's heavy atoms (like carbon or phosphorus), that water molecule was removed to avoid overlapping. Sodium and chloride ions were then added to neutralize the structures inside the water boxes to achieve a total concentration of approximately 50 mM of ions. Neutralization is meant to achieve physiological conditions as molecules can have inherent overall charges, which are balanced to no charge when present in cytoplasm or blood serum. Additionally, ion concentrations within the box were meant to represent the charges within cells and blood serum. An example of a neutralized EBNA1 structure is shown in **Figure 2**.

The structures were then forced to enter their most stable conformations with energy minimization of the water molecules, ions, and main structures according to Hwang et al.<sup>11</sup> This involved adding, then slowly decreasing, constraints following harmonic trends to allow for free motion. The structures were then heated from 30 Kelvin to 300 Kelvin and the structures were allowed to reach equilibrium.

Lastly, the structures were prepared for the simulations with light constraints on the backbone carbon atoms in the proteins and phosphorous atoms in the vDNA for 2 nanoseconds (ns). Initial simulations of the DNA revealed a tendency for DNA denaturing or "fraying."<sup>12</sup> The hydrogen bonds between the terminal nucle-



**Figure 2.** EBNA1 structure neutralized in a water box. EBNA1 is shown in red and vDNA in purple, light blue lines are water molecules, and orange and blue spheres are  $\text{Na}^+$  and  $\text{Cl}^-$  ions respectively.

ic acids would break and allow for successive breakage of bonds down the DNA. To combat this, distance restraints were placed on the terminal hydrogen-binding atoms involved to keep each atom at a maximum of 2.4  $\text{\AA}$  away from each other and promote binding. This would also replicate physiological conditions because vDNA is a continuous circular strand as an episome. Restraints followed Hooke's law to allow the atoms to oscillate given a defined spring constant specific to the atom type. Finally, the covalent bond lengths for hydrogen atoms were set using the SHAKE CHARMM method similar to Hwang et al.<sup>13</sup> All structures were then transferred to TAMU HPRC to perform the simulations with OMM. Positional coordinates throughout the simulations were saved every 20 picoseconds using the domain decomposition CHARMM method and all systems lasted for 200 ns with 10,000 total frames.

## RESULTS

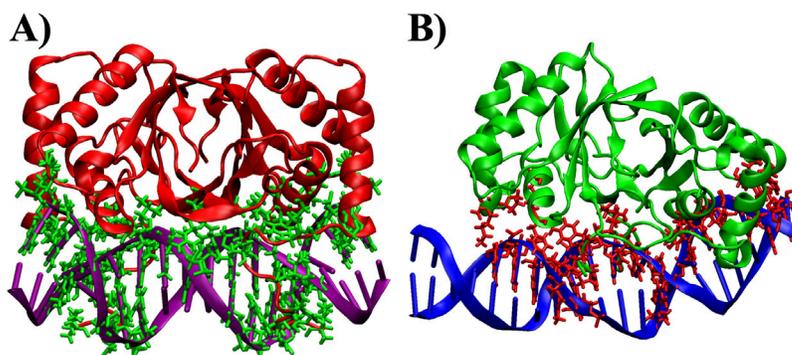
The noncovalent interactions between the atoms in the protein-vDNA complexes, including hydrogen bonds and nonpolar contacts, were calculated

according to CHARMM HBOND and previous developments by Brooks et al.<sup>14</sup> The hydrogen and nonpolar binding events throughout the simulation found EBNA1 created a larger total number of noncovalent contacts compared to LANA. Specifically, EBNA1 had 31 different hydrogen bonds and 32 nonpolar contacts while LANA had 14 hydrogen bonds and 17 nonpolar contacts. In total, EBNA1 had at most 63 bonds while LANA only had 31 bonds, which leads to the hypothesis that EBNA1 requires more RBFE. Both the hydrogen and nonpolar interactions are shown in **Figure 3** in contrasting-colored ‘sticks,’ where the difference in bond number is apparent.

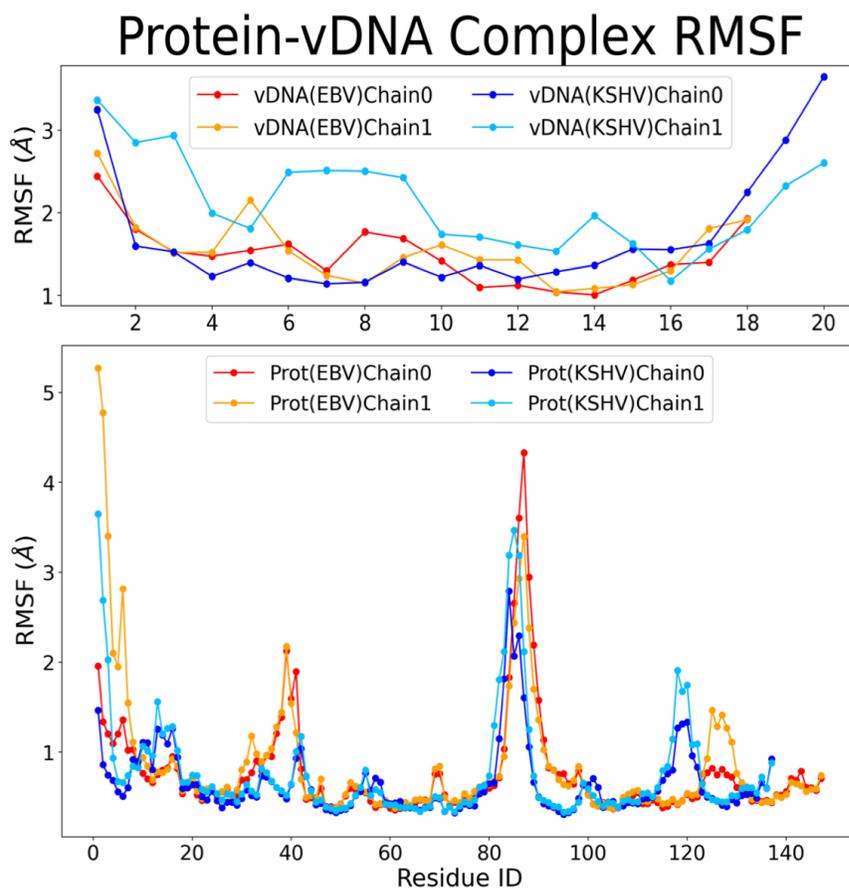
The root-mean-square fluctuation (RMSF) calculation was performed to determine which amino and nucleic acids (residues) were the most mobile relative to the reference structure. The more mobile structures will have a higher RMSF because they are not bound. The combined RMSF of the proteins and vDNAs from the two complexes can be seen in **Figure 4**, with peaks at ranges of residues where there is increased mobility and thus fewer bonds.

The root-mean-square deviation (RMSD) was calculated to find the deviation of the entire structure versus the original structure over time. Protein residues with a RMSF above 1 Å and vDNA residues with a RMSF above 2 Å were ignored for the RMSD calculation to avoid excessive noise from highly-mobile regions in the structure. The resultant RMSD plot of all structures excluding the highly mobile residues is shown in **Figure 5**, comparing the RMSD of the protein and vDNA chains from the EBNA1 and LANA complexes.

On average, the vDNA chains deviated  $3.66 \pm 0.43$  Å whereas the protein chains deviated  $0.68 \pm 0.06$  Å. This difference in movement in space can be attributed to the number of binding interactions present. The binding of the vDNA by the protein can determine if the vDNA has the ability to deviate in position. Al-



**Figure 3.** Noncovalent contacts that lasted for 40% of the simulation shown as contrasting-colored sticks. **A)** EBNA1 complex. **B)** LANA complex.



**Figure 4.** RMSF of parts of vDNA and protein in EBNA1 and LANA complexes.

though there are more noncovalent bonds for EBNA1 than LANA, the average RMSD of vDNA<sup>EBV</sup> was higher than that of vDNA<sup>KSHV</sup>.

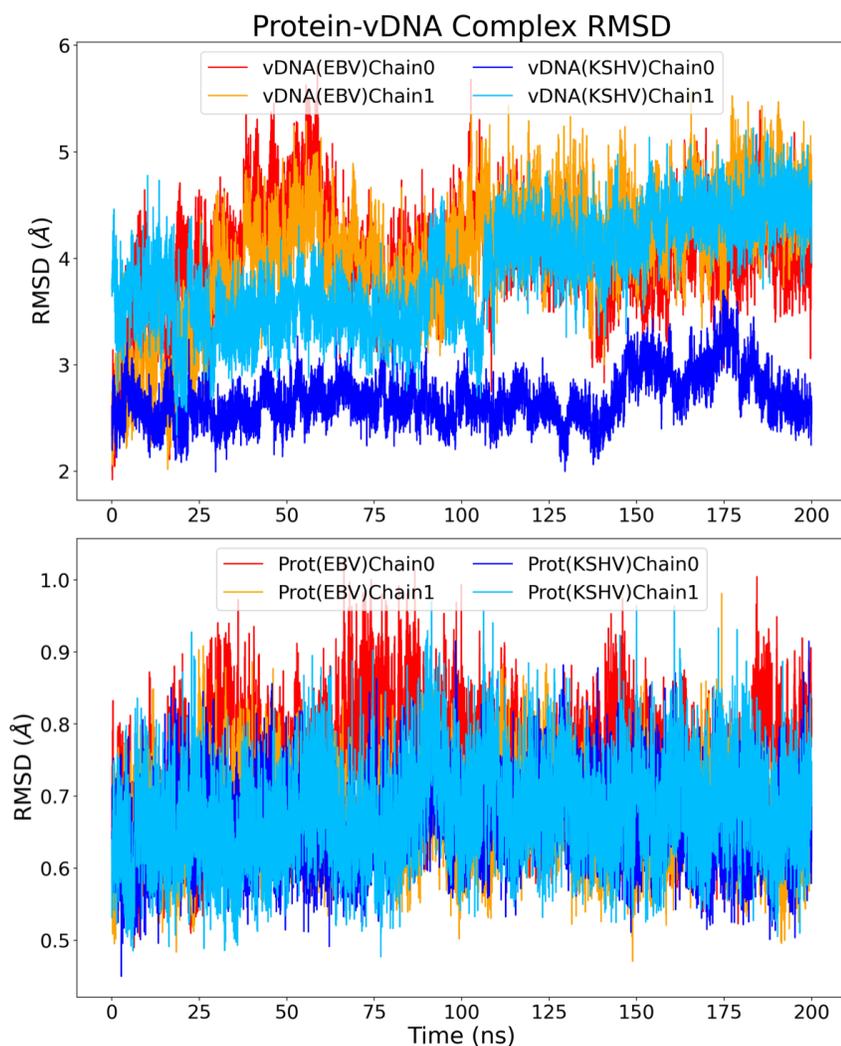
The buried surface area was computed to find the area of each structure that was used for binding. For each complex, the calculation involved adding the outer area of the protein by itself with the outer area of the vDNA by itself, then subtracting the outer area of the complex from the previous sum (**Equation 1**); where  $A_1$  is the outer area of the protein,  $A_2$  is the area of the vDNA, and  $A_{12}$  is the shared area of  $A_1$  and  $A_2$  complex. The expression is halved because the numerator results in twice the complex area, resulting in the change in area of the protein-vDNA complex binding interface. This calculation was repeated for each frame

$$\text{Buried Surface Area} = \frac{A_1 + A_2 - A_{12}}{2}$$

**Equation 1**

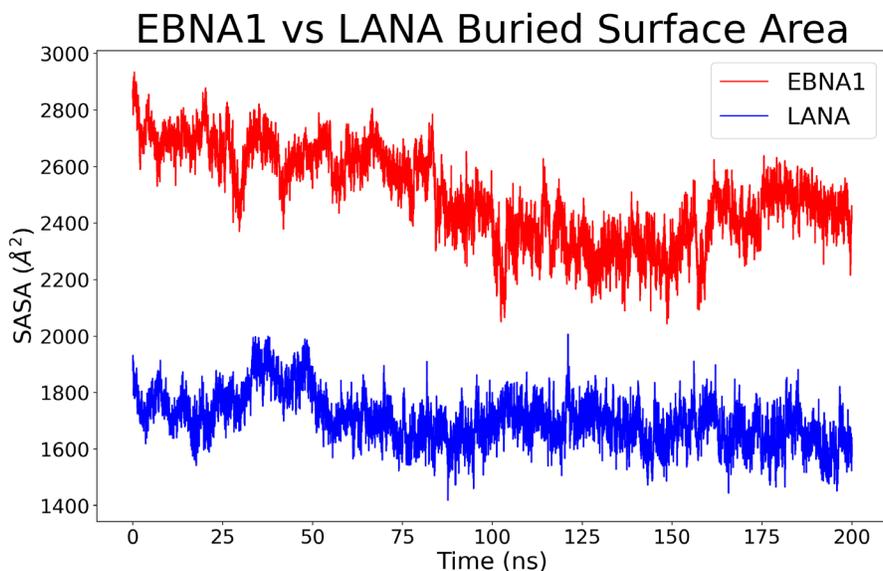
in the simulation and can be seen in **Figure 6**.

A direct correlation can be made between an increased buried surface area and increased binding energy.<sup>15</sup> The average and standard deviation area for the EBNA1 complex for the most stable last 100 ns is  $2616.90 \pm 107.73 \text{ \AA}^2$ , whereas the LANA area is  $1734.74 \pm 91.39 \text{ \AA}^2$  for the last 100ns. According to these values, it can be inferred that the EBNA1 complex has a stronger binding free energy compared to that of the LANA complex.



**Figure 5.** RMSD of whole EBNA1 and LANA structures in space over 200ns.

The conformational entropy was found using the method from Shi et al.<sup>16</sup> Their study used information theory to relate the random process, which is sidechain dynamics, to a probability distribution of how the structure is able to fluctuate in space.<sup>17</sup> The entropy was found by first calculating the dihedral angles of the backbone and sidechain atoms of both the protein and vDNA structures. This calculation finds how much each structure rotates around a specific axis defined by three consecutive bonds and the four atoms involved. To verify the accuracy of these values, the angles throughout the entire simulation were subsampled for five separate entropy calculations. Specifically, every fifth dihedral angle was separated resulting in five 40 ns measurements instead of one 200 ns measurement. From these subsampled trajectories, an average and standard deviation entropy value for each structure was obtained and plotted against the total simulated time, as shown in **Figure 7**.



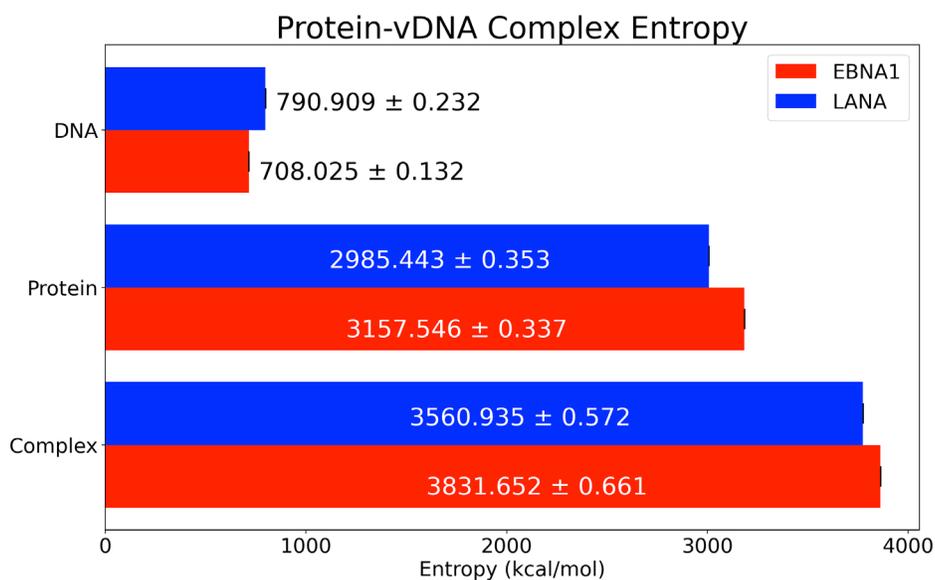
**Figure 6.** Buried Surface Area of EBNA1 and LANA protein-vDNA complexes over 200ns.

**Figure 7** depicts the conformational changes that can occur from the studied structures because of the difference in structure size. For example, the EBNA1 protein has 147 amino acids and an entropy of 3184.229 kcal/mol compared to 137 and 3007.464 kcal/mol for LANA. Similarly, the vDNA<sup>KSHV</sup> has 20 nucleic acids and an entropy of 798.504 kcal/mol, while vDNA<sup>EBV</sup> only has 18 nucleic acids and an entropy of 715.019 kcal/mol. An increase in dihedral rotation around bonds is related to an increase in the number of bonds. This relation can be seen in **Figure 3** since the EBNA1 complex creates more bonds between the protein and vDNA.

The energy of each structure as well as the solvation energy was calculated to find the relative binding free energy. Negative RBEF values correspond to complexes favoring a bound state. This was done according to the methods in Zoete et al. where the binding free energy is the sum

of the energy contributions from the van der Waals and electrostatic energies ( $\Delta G_{\text{bind}}^0$ ), the difference between the solvation energy of the complex and the sum of the two components ( $\Delta G_{\text{desolv}}$ ), and the difference of the entropy ( $-T\Delta S$ ).<sup>18</sup> The equation of the differences in solvation energy can be seen in **Equation 2**, while the binding free energy equation can be seen in **Equation 3**. The solvation energy used the Generalized Born model for Molecular Volumes (GBMV) and with a Switching function (GBSW) using CHARMM GBMV and GBSW commands.<sup>19</sup> The solvation energy

terms for the complex,  $\Delta G^D$ , and the monomers,  $\Delta G^M$ , used the calculated GBMV or GBSW for the complex and protein and vDNA from the complex trajectory, respectively. Dissimilar to **Equation 2**, the difference in entropy is calculated using the conformational entropy values from the complex, sole protein, and sole vDNA simulations.



**Figure 7.** Conformational Entropy of protein-vDNA complexes, sole proteins, and sole vDNAs.

$$\Delta G_{desolv} = \Delta G_{solv}^D - (\Delta G_{solv}^{M1} + \Delta G_{solv}^{M2})$$

**Equation 2**

$$\Delta G_{bind} = \langle \Delta G_{bind}^0 \rangle + \langle \Delta G_{desolv} \rangle - \langle T\Delta S \rangle$$

**Equation 3**

**Table 1.** GBSW RBE values for EBNA1 and LANA complexes.

Energy	EBNA1 GBSW (kcal/mol)	LANA GBSW (kcal/mol)
$\Delta G_{bind}^0$	$-9928.6 \pm 225.5$	$-5788.5 \pm 136.0$
$\Delta G_{solv}^{M1}$	$-6143.9 \pm 122.1$	$-4175.3 \pm 104.0$
$\Delta G_{solv}^{M2}$	$-12278.8 \pm 87.3$	$-14299.8 \pm 138.5$
$\Delta G_{solv}^D$	$-8756.1 \pm 140.0$	$-12823.4 \pm 156.0$
$-T\Delta S$	37.5	31.64
$\Delta G_{bind}$	$-224.6 \pm 33.1$	$-105.2 \pm 17.9$

The final GBSW RBE values are tabulated in **Table 1**, where a more negative value for  $\Delta G_{bind}$  can be associated with stronger binding free energy. As shown, the EBNA1 protein-vDNA complex elicited a lower RBE compared to LANA.

We hope the RBE values of  $-224.6 \pm 33.1$  kcal/mol for EBNA1 and  $-105.2 \pm 17.9$  kcal/mol for LANA can be used to further inhibitor development for EBV and KSHV, similar to Ancy et al.<sup>20</sup> Explicitly, potential inhibitors exhibiting similar RBE values to those reported here via MD analyses can be assumed to have a similar binding affinity to the EBNA and LANA proteins.

## CONCLUSION

The EBNA1 and LANA proteins are responsible for prolonging the longevity of infected host cells and stimulating cancer development. If the binding of these proteins to vDNA was stopped, the virus would not be able to replicate vDNA and inhibit the immune response and apoptosis. The noncovalent interactions, buried surface area, and RBE analyses support that the EBNA1 protein binds more strongly to vDNA<sup>EBV</sup>

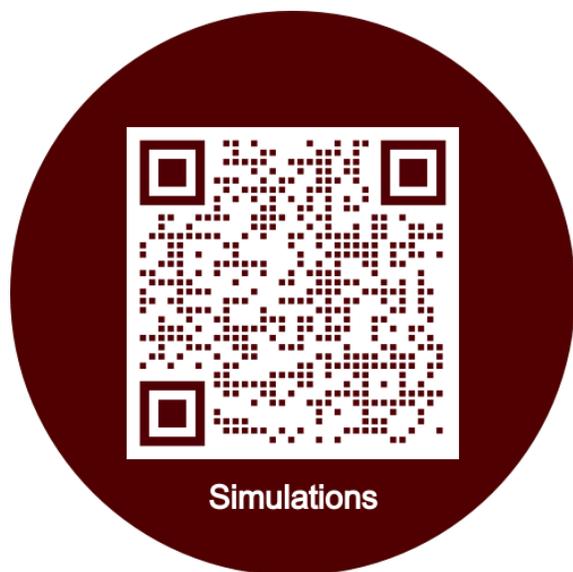
than LANA to vDNA<sup>KSHV</sup>. According to Hellert et al., EBNA1 attaches to vDNA with specific “linking domains” that are separate from the regions specific to DNA-binding.<sup>21</sup> This contrasts with how LANA uses the same region for DNA-binding and DNA tethering. With relation to RBE, this can explain the lower RBE of LANA because its binding regions are expended for tethering as well as sequence-specific vDNA-binding. Overall, we hope these calculations could be used for inhibitor development to disrupt the binding of these DNA-binding proteins to vDNA. For future studies, we could perform a docking simulation to visualize the sequence-specific binding.

## ACKNOWLEDGEMENTS

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## SUPPLEMENTAL INFORMATION

The QR code gives access to a YouTube video of the simulations performed. Each simulation has been sped up to fit within thirty seconds.



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### **SARAH E. FROSS '22**

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# Accelerating Training Through Personalization in Augmented Reality

By Isabella C. Pedron '23, Connor M. Barnes '23, Ashley E. Bailly '23



Photo by Mat Napo on Unsplash.

## INTRODUCTION

The U.S. Emergency Medical Services (EMS), composed of first responders, emergency medical technicians (EMTs), paramedics, firefighters, and others, is a vital system that provides urgent pre-hospital treatment and transport. These emergency response (ER) workers attend to over 42.5 million calls every year.<sup>1</sup> However, career burnout and relatively low salaries have led to a significantly high annual turnover rate of 20–30%, with estimated turnover costs being \$6,400 and \$7,700 per full-time EMT and paramedic, respectively.<sup>2</sup> In recent years, these issues have caused an acute nationwide shortage of ER workers, causing slower response times, a lack of services, and a deficit of supplies in many areas.

The ongoing COVID-19 pandemic has caused the ER worker shortage to reach a crisis level. ER training programs were paused or shut down entirely, the National Registry of EMTs stopped its certification testing, and hospitals short on nursing staff began to hire paramedics at higher wages. These workforce shifts occurred at a time when the nation saw an unprecedented increase in EMS call volumes.<sup>3</sup> There is a pressing need to expedite the training of ER workers while maintaining high quality of instruction.

To address this need, we collaborated with several other universities and public safety partners to develop an adaptive EMS training platform called LEARNER. LEARNER integrates modern human aug-



Scan the QR code to watch the LEARNER project video created by the NeuroErgonomics Lab, or visit <https://tx.ag/509LEARNERvideo>.

**BLENDING REAL AND VIRTUAL CONTENT OFFERS UNIQUE BENEFITS OVER OTHER TRAINING METHODS BY CREATING MORE IMMERSIVE AND REALISTIC LEARNING EXPERIENCES.**

mentation technologies such as robotics, virtual reality (VR), and augmented reality (AR), to improve work conditions of EMS personnel as well as provide immersive and accessible modalities of training. Our goal is to accelerate expertise development by implementing an innovative approach for EMS-based curriculums by tailoring training to individual program and trainee needs. Our latest study focuses on using AR as a training delivery method.

AR technology superimposes virtual objects on the real world. Blending real and virtual content offers unique benefits over other training methods by creating more immersive and realistic learning experiences. Other benefits of AR include the ability to program a wide variety of customized scenarios for training and its high level of portability, as it only requires a head mounted device or a smartphone with an AR application.<sup>4</sup> These features enable greater levels of personalization and allow for situational learning that fits the user's needs, which have been shown to make the learning process easier and faster. In fact, AR-based training is already used in a variety of medical applications, such as surgical training, and has reduced the amount of time needed to practice certain skills.<sup>5</sup> Even so, there is little empirical evidence regarding the effectiveness of this emerging technology.

In a previous study conducted by our lab, we compared the efficacy of AR-based training to video-based training, a more conventional training method. We analyzed how first-time AR users learned how to interact with the AR interface in different training

modalities by evaluating parameters that affected performance during training and retention. We found that AR-based training resulted in better immersion and higher user engagement; however, it was associated with greater frustration, lower focused attention, and higher cognitive load.

Building upon the aforementioned research, this current study aims to: 1) evaluate a machine learning algorithm designed to advance the development of a personalized training program while taking into consideration the drawbacks of AR-based training previously found; and 2) examine differences in subjective, physiological, and performance metrics between high and low performers.

## METHODS

### Participants

We recruited 12 participants (50% females) for our study with a mean age of  $21 \pm 1$  years. Recruitment was limited to right-handed individuals with less than one hour of AR/VR experience. These requirements ensured that all participants used the same, dominant hand for the duration of the study and began with the same limited knowledge of immersive, interactive environments. For safety reasons, additional exclusion criteria included an allergy to adhesives, a history of photosensitive epilepsy or seizures, and a metal implant

or other device in the head. Participants were compensated for their time. This research was approved by the Institutional Review Board (IRB2021-0742DCR).

### Study Protocol

Each AR session was completed in one day, lasting about two and a half hours. The study had four phases: setup, AR familiarization, AR adaptation (if needed), and evaluation. **Figure 1** summarizes the study protocol.

At the beginning of each session, participants were outfitted with an Actiheart device (Actiheart 4, CamNTEch, Inc., UK) to collect heart rate data, an Empatica watch (Empatica E4, Empatica Inc., USA) to collect electrodermal activity data, and an AR headset (Microsoft HoloLens 2). We collected initial subjective surveys and baseline data for three minutes prior to AR training.

During familiarization, participants learned four AR interaction tasks: 1) poking, 2) raycasting, 3) scrolling, and 4) moving. The order of tasks was varied to reduce order bias. Participants trained on the four tasks by completing six trials for each task. After each task, subjective surveys were collected regarding the participant’s experience. **Figure 2** showcases these tasks in AR via video.

1. **Poking** involved pointer finger selection and was utilized when the virtual elements were

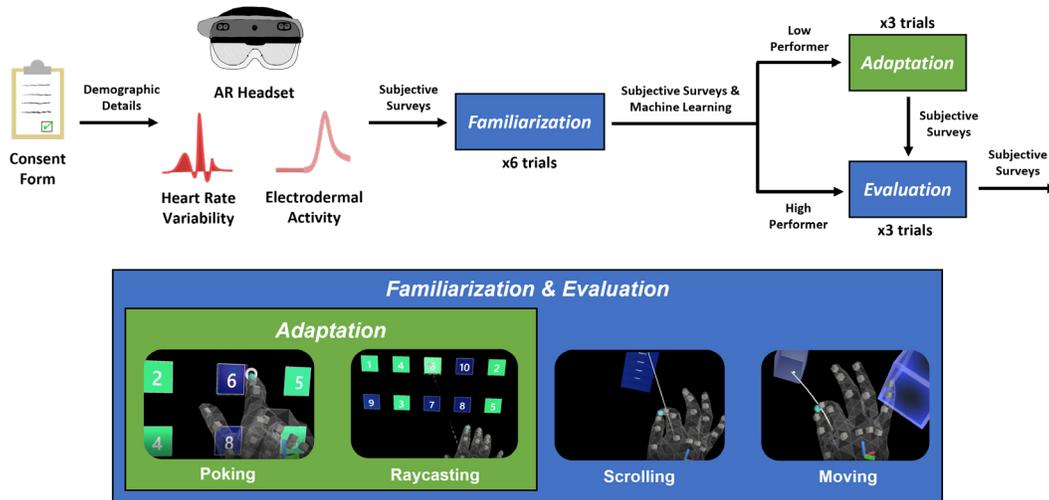


Figure 1. Study protocol flowchart.

within arm's reach of the user. The user was instructed to select the numbers one through ten in ascending order.

2. **Raycasting** was utilized when the virtual elements were out of arm's reach of the user. This selection method required the user to direct a beam of light from their palm and then select elements by pinching their pointer finger and thumb together. The user was again instructed to select the numbers one through ten in ascending order.
3. **Scrolling** involved scrolling through a list, requiring the user to direct a beam of light from their palm, grab elements by pinching their pointer finger and thumb together, and scroll up or down by moving their hand vertically. During this task, the user selected a certain word from a menu.
4. **Moving** involved moving an object from one location to another. The user performed this by pinching their pointer finger and thumb together, moving their hand horizontally, and then releasing their fingers.

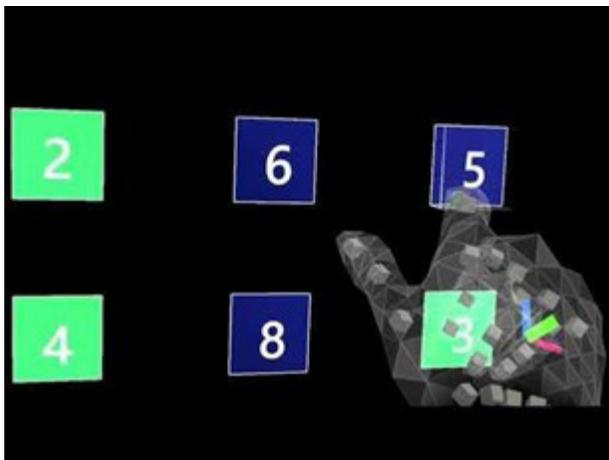
Next, the participant's AR interaction data was run through our machine learning algorithm to determine whether adaptation (extra practice) was needed. Only poking and raycasting were considered for

adaptation as raycasting was a prerequisite to perform scrolling and moving. If the machine learning algorithm deemed extra practice was needed for poking and/or raycasting, the participant would undergo additional training by completing three more trials for the task(s).

Lastly, the evaluation phase gauged the participant's success at learning the AR tasks by repeating all four tasks again for three trials each.

### *Adaptation Model*

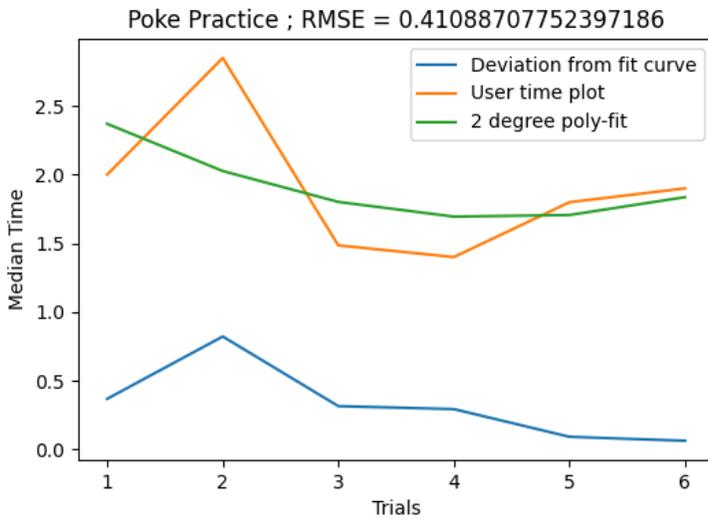
We analyzed two metrics from twenty-seven participants from our prior study as measures of performance and consistency, respectively: 1) total median time<sup>6</sup> and 2) root mean square error (RMSE), a measure of distance from a prediction.<sup>7</sup> Total median time was calculated by taking the median of the overall time required for each trial and adding all the median values. RMSE was calculated by fitting a 2-degree polynomial over the learning curve comprising performance and number of trials, as seen in **Figure 3**. We used k-means clustering with two clusters of high and low performers, as portrayed in **Figure 4**. Low performers were designated as those who had a higher median time and RMSE. New participant data points were excluded from the original dataset to prevent changes in the cluster centroid coordinates. The new participant data was compared to these high and low performer centroid coordinates to determine if adaptation was needed.



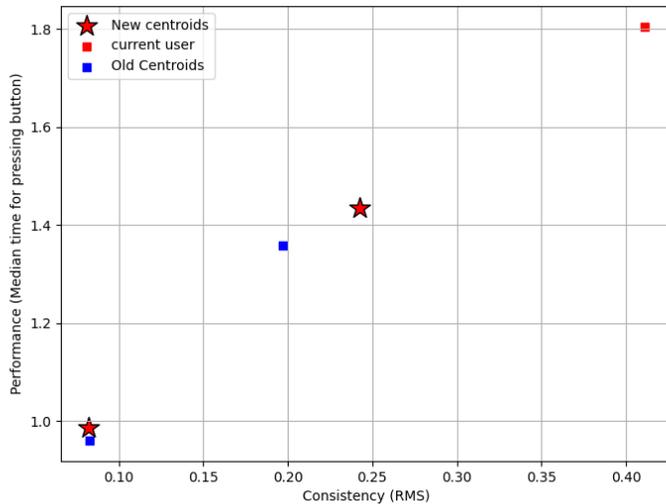
**Figure 2.** Video of the four augmented reality tasks: poking, raycasting, scrolling, and moving.



Scan the QR code to watch the Figure 2 video, or visit <https://tx.ag/509Fig2>.



**Figure 3.** Plot of median time and trials for RMSE of a skill.



**Figure 4.** Plot of performance vs. consistency of a skill.

## Measurements

### Subjective Measures

According to Cognitive Load Theory (CLT), an individual's performance is limited by the capacity of their working memory. CLT subdivides different loads into three categories: intrinsic, germane, and extraneous. Intrinsic is associated with the specific task, germane refers to the construction of long-term memories, and extrinsic is based on how the task is presented.<sup>8</sup> Germane cognitive load is independent of materials presented, but intrinsic and extraneous are directly

affected by the interactivity and complexity of tasks. For these reasons, intrinsic and extraneous loads are useful metrics in this study.<sup>9</sup> To assess the cognitive loads of participants, a standard CLT questionnaire was administered after each task. Each measure of CLT is on a one to seven scale, where seven represents a high cognitive load. An additional question asked each participant if they believed they needed more practice to obtain each new skill.

### Physiological Measures

Electrocardiogram (ECG) and electrodermal activity (EDA) physiological parameters were measured as part of quantifying cognitive load. Research has shown ECG signals to be an important cognitive load indicator by analyzing heart rate (HR)<sup>10</sup> and heart rate variability (HRV).<sup>11</sup> A higher cognitive load causes the heart to beat faster.<sup>12</sup> HRV is a quantitative marker for autonomic nervous system activity by describing the fluctuation in time between consecutive heartbeats. Two HRV features are commonly extracted to detect variations in cognitive load. The root mean square of successive differences (RMSSD) reflects the beat-to-beat variance in HR, and a higher cognitive load results in a lower difference.<sup>13</sup> The low-to-high frequency ratio (LF/HF) is calculated using LF and HF measures which reflect stress and recovery, respectively, and a higher cognitive load results in a higher ratio.<sup>14</sup>

EDA, the measure of skin electrical conductance or sweat gland activity, has also commonly been used as a method for determining cognitive load.<sup>15</sup> EDA is divided into two phenomena: 1) a tonic component defined as electrodermal level which represents slow-changing, general characteristics of the signal and 2) a phasic component defined as electrodermal response which represents faster-changing elements, including responses from specific stimuli.<sup>16</sup> Due to the nature of the signal, the sampling rate and duration of data collection are vital to consider during experimental design; however, both served as limitations during

analysis.

## Statistical Analysis

Analysis of Variance (ANOVA) is a tool used in statistics to determine if experimental results are significantly different from a control or between groups. The probability value ( $p$ -value) represents the probability of the occurrence of a value at least as extreme assuming that there is no real difference. The lower the  $p$ -value, the more significant a result is. Thus, a series of two-way ANOVA tests were performed to determine the impact of the study phases and need for adaptation on the subjective, physiological, and performance measures. The criteria used to find the statistical main effect of the dependent variable on the independent variable was a  $p$ -value  $< 0.05$ .

## RESULTS

Only poking and raycasting were analyzed since these were the only tasks considered for adaptation. Physiological data was normalized to the participant's baseline data to rule out individual differences. To evaluate the effectiveness of the adaptation model, participants were divided into two groups: low performers who needed adaptation ( $n = 7$ ) and high performers who did not need adaptation ( $n = 5$ ). Analysis was also performed across the phases of familiarization and evaluation.

### Subjective Measures

For poking, no significant differences were

found between groups or phases in either extraneous or intrinsic loads. For raycasting, there was no significant difference found between groups in intrinsic or extraneous load. However, intrinsic load showed a significant difference between phases ( $p = 0.047$ ) and extraneous load showed a significant difference between the two phases ( $p = 0.016$ ). In other words, lower cognitive loads were reported between familiarization and evaluation for the raycasting task.

Furthermore, although extra training for each participant was determined by clustering with high and low performers, each participant was asked if they believed they needed more training to acquire the skill. From the survey, 70% of participants agreed with the model, 20% believed that they had acquired it, but the model determined that they needed more practice, and the remaining 10% answered they needed more practice, but the model determined they did not.

### Physiological Responses

For poking, we found a statistically significant difference in HR by both the phase ( $p < 0.001$ ) and group ( $p < 0.001$ ), though the interaction between these terms was not significant. There were no statistically significant differences in RMSSD or LF/HF by either phase, group, or interaction between these terms; however, there was a trend toward significance in RMSSD by phase ( $p = 0.085$ ).

For raycasting, there were no statistically significant differences in HR, RMSSD, or LF/HF by either phase, group, or interaction between these terms;

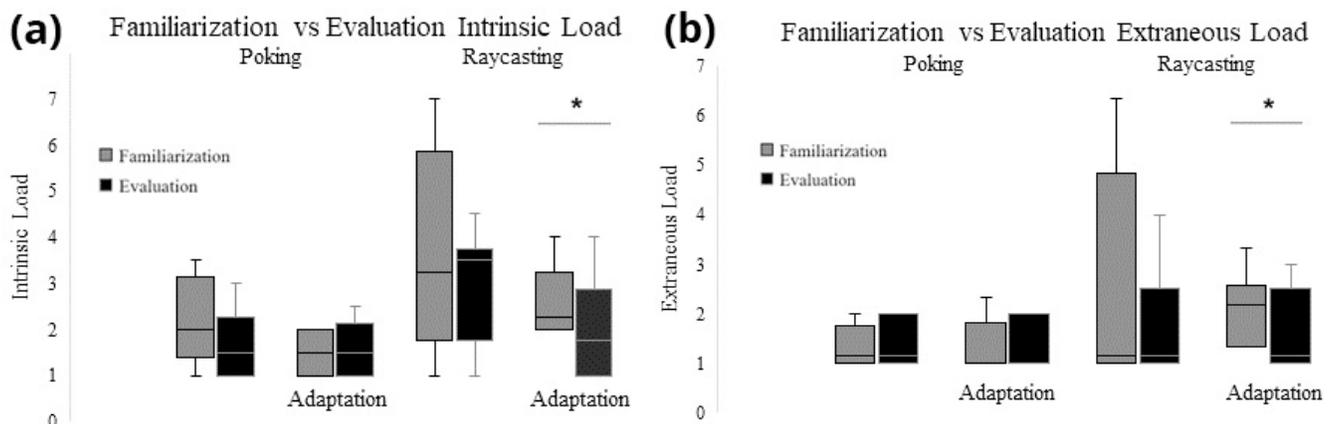
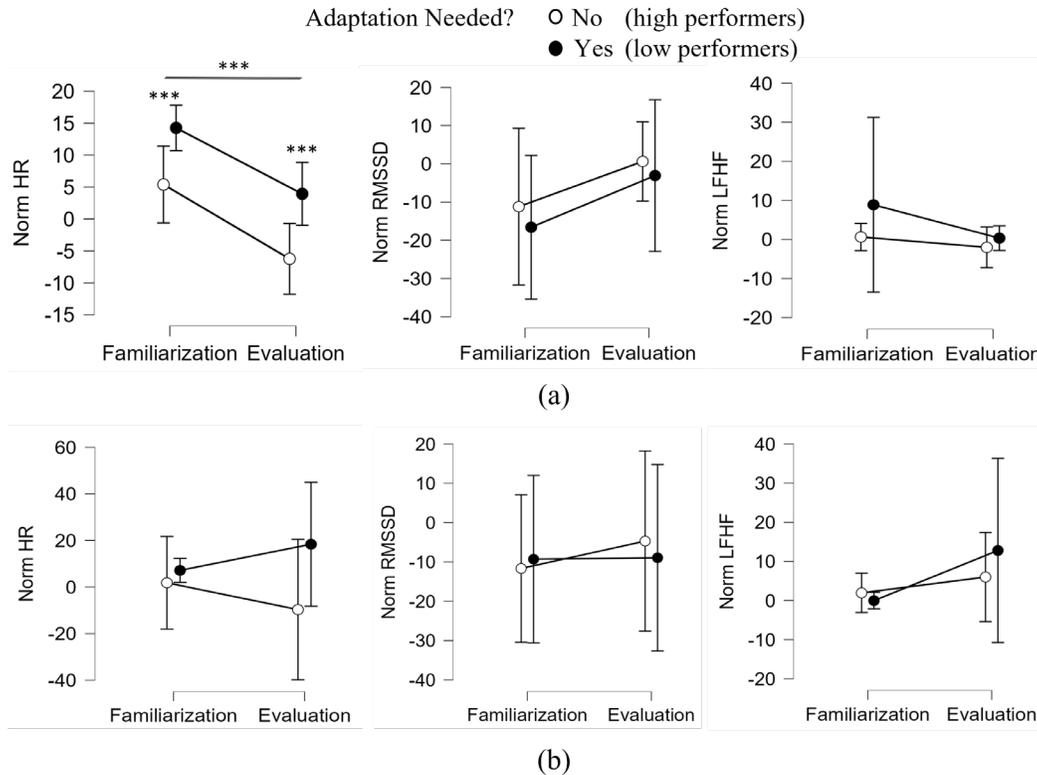


Figure 5. Box plots for (a) intrinsic load and (b) extrinsic load across tasks, phases, and groups.



**Figure 6.** Normalized ECG for (a) poking and (b) raycasting tasks.

however, there was a borderline of significance in HR by group ( $p = 0.059$ ).

While pre-processing the EDA data, initial analysis revealed that the quality of the obtained data was not adequate to determine viable results. For future studies, it should be noted that the sampling rate should be increased due to the temporal sensitivity of the data, and the length of recording time should be increased to obtain enough relevant data due to the slow measure nature of the signal.

### Performance

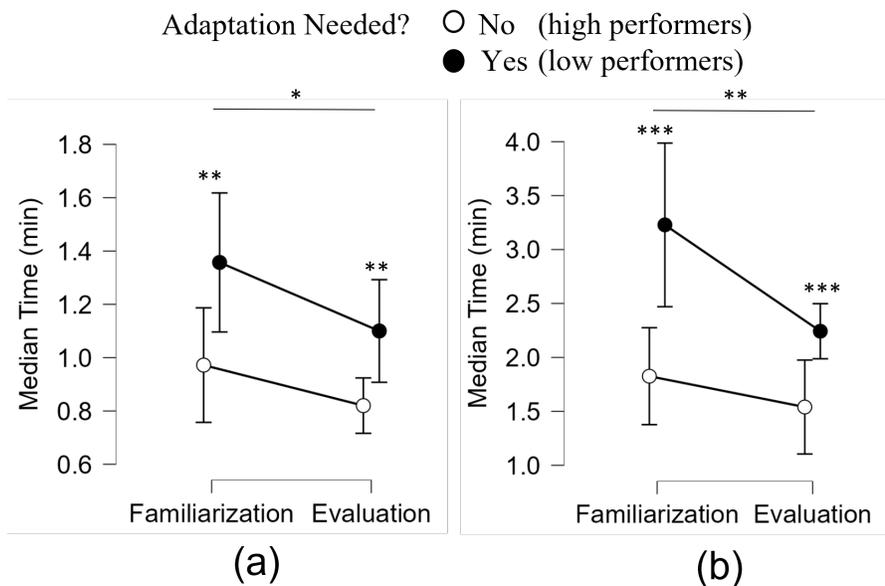
For both poking and raycasting, we found a statistically significant difference in median time by both the phase ( $p = 0.029$ ;  $p = 0.008$ ) and group ( $p = 0.001$ ;  $p < 0.001$ ), though the interaction between these terms was not significant.

When comparing median time from familiarization to evaluation, the low performers who required adaptation on poking and/or raycasting were on average 17.8% and 37% quicker, respectively. However,

the high performers who did not receive extra practice on poking or raycasting were on average 10.2% and 16.21% quicker, respectively.

## DISCUSSION

This study evaluated a machine learning algorithm method to personalize AR training and studied differences between high and low performers. Preliminary results show that intrinsic and extraneous loads are mainly unaffected by a participant's performance categorization. The only significant difference in subjective cognitive load was in the adaptation group having a decreased load, both intrinsic and extraneous, in the raycasting task. This may mean that the adaptation protocol is able to reduce cognitive load in certain tasks, but not others. Furthermore, the protocol identified 30% of cases where an individual's subjective judgment did not match the recommendation of the model. If the choice for further training was left up to the participants, 20% would be under-trained on the system and 10% would spend longer than necessary on



**Figure 7.** Performance for (a) poking and (b) raycasting tasks.

their training.

The ECG data suggests that low performers consistently sustained a higher cognitive load compared to high performers. For poking, participants who needed adaptation experienced a higher cognitive load compared to participants who did not need adaptation as seen from the significantly higher HR. This may be a consequence of fatigue from extra practice or the fact that the non-adaptation group consisted of high performers, as deemed by the machine learning algorithm. The raycasting task revealed unexpected results when analyzed by phases. The adaptation group generally experienced an increase in cognitive load between phases as seen from the trending increase in HR and increase in LF/HF.

The overall performance indicates that the adaptation model was effective at personalizing training. Looking at familiarization, performance showed that the machine learning algorithm successfully predicted whether a user was a high or low performer. Furthermore, low performers improved their median time more than high performers after undergoing additional training, suggesting the extra practice protocol was productive.

One limitation of this study was the small sample size. Statistical power is limited with small

sample sizes, so unsurprisingly many of the tests failed to show significance due to a high standard deviation. Another limitation was the extremely short time duration for the AR tasks due to the reduced number of trials. The reduced number of trials was done to minimize fatigue and optimize performance, but a consequence is that the physiological data may not be reliable to analyze as they are slow changing measures.

The next steps should be to solidify our findings by conducting more studies, increasing the sampling rate, and analyzing other types of physiological data

that also analyze cognitive load for a more multimodal approach. One example of this includes utilizing functional near-infrared spectroscopy (fNIRS) to map brain activity through light reflectance by oxygenated and deoxygenated hemoglobin. This analysis would showcase which areas of the brain are most active during certain AR training activities, allowing for another approach in quantifying cognitive load.

These findings will be important in the development of future adaptive training programs that will hinge on these foundational AR interactions. If our analysis can discern that user-tailored AR training is effective at reducing cognitive load and maximizing

**THE ECG DATA SUGGESTS THAT LOW PERFORMERS CONSISTENTLY SUSTAINED A HIGHER COGNITIVE LOAD COMPARED TO HIGH PERFORMERS.**

performance, future AR training platforms could prove to decrease costs and increase productivity for emergency response training. With an improved method of training based in AR, the field of emergency medicine may be better able to curb the shortage of workers and better address the limitations of current training. In the end, this may mean a safer and more productive EMS workforce.

## ACKNOWLEDGEMENTS

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# Development of a Computational Method to Analyze Mitochondrial Calcium Signals in Astrocytes

*By Maray V. Valle '22*



*Photo by Milad Fakurian on Unsplash.*

## INTRODUCTION

Astrocytes are the most abundant non-neuronal cells in the brain. Morphologically, they are composed of a cell body with thick and thin processes protruding into their astrocytic territories. Through their processes, astrocytes form connections that allow them to communicate with and regulate the functions of neurons. Until recently, astrocytes were thought to merely serve as supporting cells. However, research shows that astrocytes serve important roles in energy storage and synapse modulation, among other functions.<sup>1</sup>

Unlike neurons, astrocytes do not propagate electrical impulses called action potentials. Instead, astrocytic communication occurs through calcium ( $\text{Ca}^{2+}$ ) signals which travel to neighboring astrocytes and neurons via gap junctions, allowing ion transfer between cells.<sup>2</sup> Some of these  $\text{Ca}^{2+}$  events are almost as rapid as the firing rate of some neurons, which indicates that astrocytes sense and respond to a diverse range of neuronal signals.<sup>3</sup> Studying calcium signals is therefore of utmost importance to analyze astrocytic behavior and function, as well as to comprehend the effect astrocytes have on neurons.

Among the diverse calcium signaling events, mitochondrial  $\text{Ca}^{2+}$  signals account for a significant proportion of the total number of  $\text{Ca}^{2+}$  signals in astrocytes. Our work shows that astrocytic mitochondria have spontaneous  $\text{Ca}^{2+}$  influxes that may influence critical aspects of neuronal function. A 2018 study found that mitochondria encountered in the processes of astrocytes contribute to the local  $\text{Ca}^{2+}$  signaling and likely play a role in local ATP production and metabolism.<sup>4</sup> The influx and efflux of  $\text{Ca}^{2+}$  from mitochondria is crucial for processes like cellular necrosis and triggering neuronal firing. Mitochondrial dysfunction has been identified as a common central pathway leading to sporadic and genetic Parkinson's Disease, as well as to an increase of neuronal death and a reduction of astrocytic generation.<sup>5,6</sup> Hence, healthy mitochondria play a vital role in the overall function of astrocytes regarding successful astrocyte-astrocyte and neuron-astrocyte communication.

## MITOCHONDRIAL DYSFUNCTION HAS BEEN IDENTIFIED AS A COMMON CENTRAL PATHWAY LEADING TO SPORADIC AND GENETIC PARKINSON'S DISEASE

As crucial as the study of these mitochondrial  $\text{Ca}^{2+}$  signals are, their analysis is extremely time consuming and inefficient. Current programs that enable semi-automated analysis are functional only for certain parts of the data processing.<sup>7</sup> These programs focus on neuronal firing patterns, which tend to be less sporadic and more rhythmic. To solve this problem, we generated Measurement of Astrocytic Related Signals (M.A.R.S.), a MATLAB based computational program that automates the process of baseline correction, event detection, calculation of duration, amplitude, and frequency of each astrocytic calcium signaling event. This program allows us to look at the kinetics, location, influx, and efflux of  $\text{Ca}^{2+}$  in astrocytic mitochondria. Further generation of a spatiotemporal map will allow for identification of a relationship of the location and timing of  $\text{Ca}^{2+}$  signals. Automatization of this analysis will impact the way we study and understand astrocytes, their functions, and the role they play in the overall brain's health.

## METHODS

Data for analysis was previously generated using the mouse strain C57BL/6 WT, as described by Huntington and Srinivasan.<sup>8</sup> Eight-week-old female and male mice were housed on a 12-hour light/dark schedule and given free access to food and water. Mice were injected with an adeno associated virus 2/5 GfaABC1D-mito7-GCaMP6f to tag the areas expressing calcium, and  $\text{Ca}^{2+}$  imaged three weeks later. All experimental procedures with mice were conducted in accordance with Texas A&M University IACUC guidelines.

An Olympus FV1200 upright laser-scanning confocal microscope with 40x water immersion objective lens, a numerical aperture (NA) of 0.8, 488 nm, and 633 nm laser lines was used for slice imaging. For visualization of GCaMP6f fluorescence, the 488 nm line intensity was set at 10% maximum output, and for visualization of MitoTracker Deep Red, the 633 nm laser line intensity was set at 1-5% maximum. Other parameters, including high voltage, gain, offset, laser power, and aperture diameter, were maintained for all GCaMP6f imaging and optimized for MTDR during each session. Videos of Ca<sup>2+</sup> events were acquired at either 800 msec/frame or 1 frame/sec (FPS).

The GECIQuant program acts as an extension of the image processor, *ImageJ*.<sup>9</sup> GECIQuant allows the user to detect regions of interest (ROIs) that contain fluorescence based Ca<sup>2+</sup> signals.<sup>10</sup> The program subjects each stack of images to three modules. The first module is responsible for background subtraction to improve background noise. The second module sets up a threshold by identifying the maximum intensity image using already established methods in *ImageJ*. The third is responsible for measuring signal intensity, area, and spatial criteria of each ROI. Once the stack of images is imported into the program, the user has the option of selecting the area within the stack that represents background noise. GECIQuant then propagates the area of the demarcated section and averages the pixel intensity which is subtracted from the total number of pixels in the frame. The resulting image with the corrected background is ready for the next step, ROI detection.

ROI detection compresses the stack of images into a maximum intensity z-projection image. Using existing features in *ImageJ*, useful signal intensities were extracted from the background and classified as objects. An area criterion was applied to the image to determine the relevant Ca<sup>2+</sup> signals that met the criteria and to trace their borders. The selected signals were then subjected to further analysis.

The final step of analysis was the ROI feature extraction. First, the Ca<sup>2+</sup> signal intensities were calculated for each frame and added to the result section of the program. Then the area of each ROI

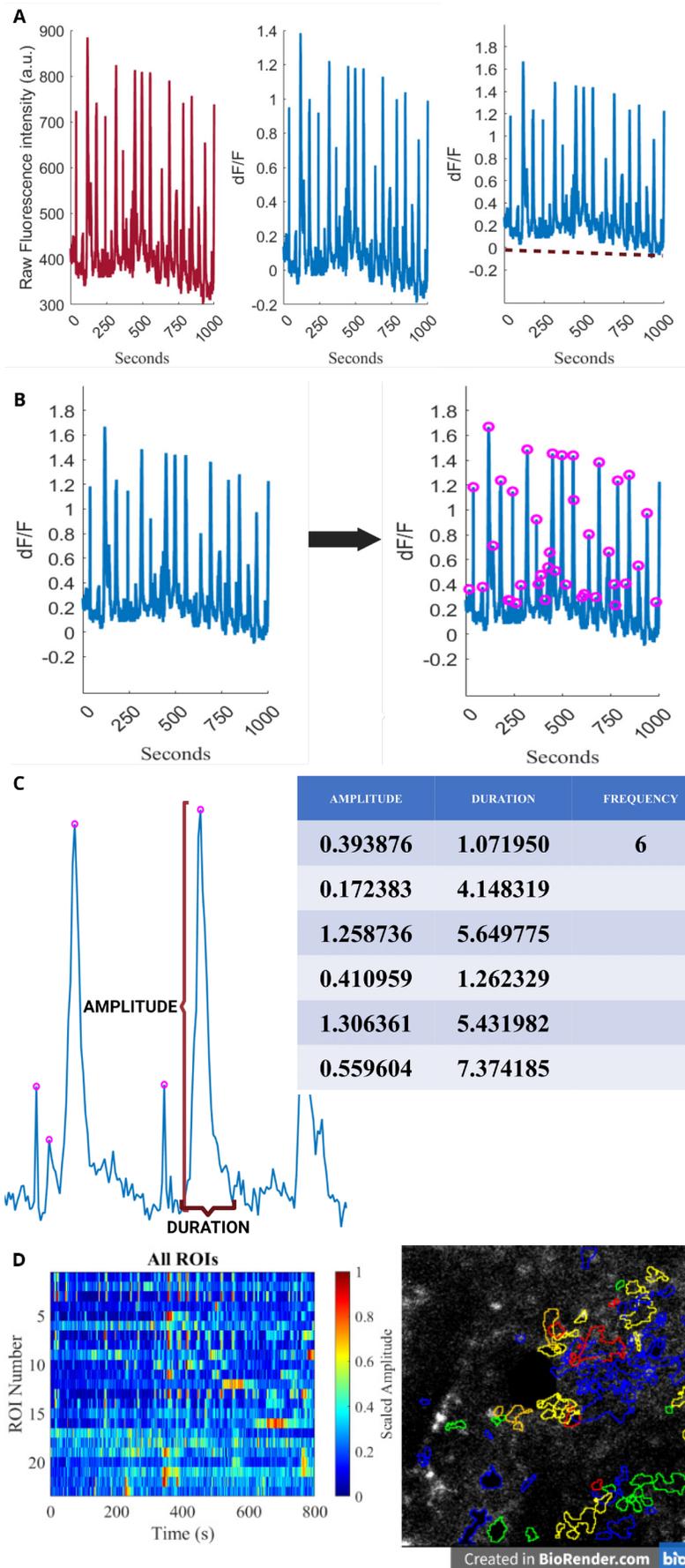
was calculated by a summation of the pixels times the pixel calibrations. The centroid distance was calculated as the average of the pixel coordinates. The data was then exported and ready for any further evaluation by MiniAnalysis.

MiniAnalysis identifies peaks of any type and size with a detection algorithm. The data acquired through GECIQuant was imported into the MiniAnalysis software which generates graphs of each ROI with a corrected baseline. The user then manually selected peaks that were considered events, those two standard deviations higher than the baseline, within each graph. After peak selection was completed, the program then calculated amplitude, frequency, and duration of each event and exported the data to an excel spreadsheet, where the data was divided into spontaneous and drug-induced calcium signals.

Once astrocyte images with GCaMP6f fluorescence were processed with MiniAnalysis, data of the GCaMP6f intensity (a.u.) across each ROI were exported to an excel spreadsheet. Each column of the spreadsheet represents one ROI, and each row represents the frame number. We input the spreadsheet into M.A.R.S which, as illustrated in **Figure 1**, processes the data by ROIs and generates a graph for each column. The x-axis of the graph shows the frame number, and the y-axis represents the GCaMP6f intensity of each ROI. Due to the randomness of astrocytic signaling, as well as drift while imaging, some of the graphs require baseline correction.

The frame corresponding to the minimum value of each ROI was identified and set apart with the next 10 frames in the column as a new array, or collection of values. This array was then subtracted from the original baseline to form a new baseline, denominated Baseline<sub>i</sub>. The mean of Baseline<sub>i</sub> was calculated, and the resulting value was used to calculate the final baseline (Baseline<sub>f</sub>), as shown in **Equations 1 and 2**.

After the Baseline<sub>f</sub> calculation, a window displays the original graph and a graph with the corrected baseline plotted side by side (**Figure 1A**). A semi-automated checkpoint allows the user to determine whether another baseline correction iteration is needed. If so,



**Figure 1.** M.A.R.S. features. Panels in the figure above illustrate M.A.R.S. components. **(A)** The automated generation of  $dF/F$  graphs and user-approved baseline corrected graph. **(B)** The process of calcium event detection on the approved  $dF/F$  graph. **(C)** The automated calculation of determined kinetic measurements, amplitude, frequency, and duration. **(D)** Shows the spatiotemporal and heat map generation of activity within an astrocyte.

$$Baseline_i = Baseline_o - \mu_{array}$$

**Equation 1**

the process is repeated with the next minimum value in the pool of frames.

After baseline correction approval, the code then proceeded to classify calcium events (**Figure 1B**). We set the parameters for events as those peaks that had two times higher standard deviation than those peaks set as a threshold. Following event detection, we processed the data for the final step in the analysis, frequency, amplitude, and duration calculation (**Figure 1C**). Amplitude was defined as the maximum extent of a  $Ca^{2+}$  event, measured from the baseline. Frequency refers to the rate at which the  $Ca^{2+}$  events were repeated over the frame period. Finally, duration refers to the extent of time each  $Ca^{2+}$  event took to reach completion.

To quantify amplitude, frequency, and duration using MATLAB., version R2021a (Massachusetts: The MathWorks Inc.), we utilized three functions that are available as part of the MATLAB package. Those functions are *findpeaks*, *MinPeakProminence*, and *half-height*. Data obtained using these functions was then saved as spreadsheets compatible with Microsoft Excel.

## RESULTS

Measurement of amplitude of calcium signals is dependent on accurate measurement of baseline fluorescence. M.A.R.S. makes baseline correction a manual checkpoint where the original graph and a baseline-corrected dF/F graph are displayed side by side and the user is prompted to select whether another iteration of baseline correction is necessary or not, as shown in **Figure 3**. If an additional iteration is needed, the code proceeds to take the next minimum value en-

$$Baseline_f = \frac{Baseline_o - \mu_{Baseline_i}}{\mu_{Baseline_i}}$$

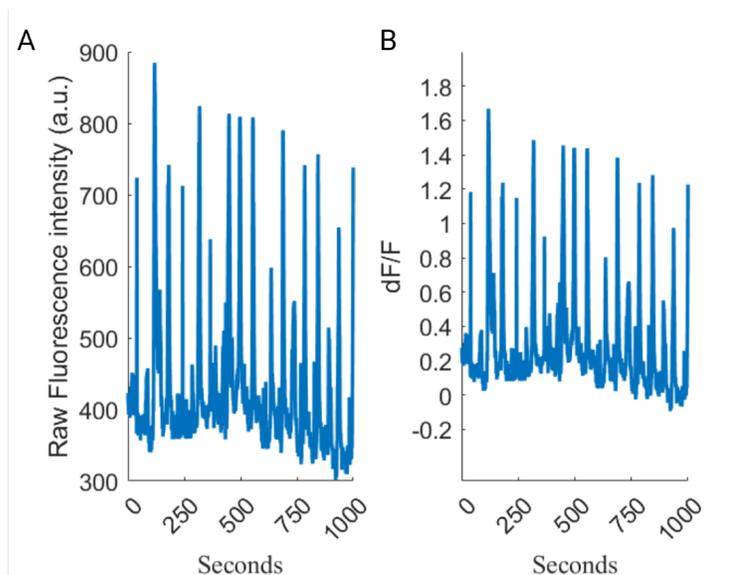
**Equation 2**

countered in the original data and employ **Equations 1** and **2** to calculate a new baseline and display the new graph next to the first dF/F graph, as illustrated in **Figure 2**. Once the baseline is user-approved, the program classifies events and obtains their corresponding kinetic measurements, amplitude, duration, and frequency.

Introducing this manual checkpoint allowed for more accurate detection of events since with each iteration a new minimum value was chosen and a new threshold was set. We observed a difference of +/- 10 events with different iterations of baseline-corrections from the same graphs. This makes the M.A.R.S. a more sensitive method for event detection.

### *Measurement of Spontaneous Calcium Dynamics using M.A.R.S.*

The measurement of calcium dynamics in vivo and in astrocytes is an important way to understand calcium biology. We first sought to determine if M.A.R.S.



**Figure 2.** Baseline correction. **(A)** Graph A illustrates the original raw data from the spreadsheet. **(B)** Graph B plots the dF/F graph with a corrected baseline calculated with Equations 1 and 2 described in the Methods section.

Is the baseline corrected? [Y/N] N

ans =

159.4350

**Figure 3.** Manual checkpoint window. Message depicted in the picture allows the user to determine whether another baseline correction iteration is needed. The numerical value provided represents the frame number at which minimum value used for baseline-calculation is encountered in the original data.

provides reliable measurements of amplitude, frequency, and duration of  $Ca^{2+}$  signals. To determine the validity of calcium signals analysis obtained from our M.A.R.S., we compared it with data previously obtained using MiniAnalysis. For the first parameter of comparison, we looked at spontaneous peaks, which were classified as those events that would occur before the 300 frames. **Figure 4** illustrates the kinetic measurements of two astrocytes obtained with both M.A.R.S. and MiniAnalysis. When the average values for am-

		MiniAnalysis			M.A.R.S.		
		Amplitude	Duration	Frequency	Amplitude	Duration	Frequency
Cell 1 <sub>n=8</sub>	Soma	0.19	2.43	2.75	0.19	2.66	2.10
	Large Territory	0.11	2.49	1.75	0.12	2.30	1.27
	Small Territory	0.36	2.28	3.25	0.31	2.66	3.90
Cell 2 <sub>n=4</sub>	Soma	0.49	3.32	2.50	0.48	3.58	2.30
	Large Territory	0.06	3.32	1.50	0.07	3.26	1.90
	Small Territory	0.23	1.88	2.25	0.24	1.80	2.35

**Figure 4.** The data represents average values of each of the kinetic parameters, amplitude, duration, and frequency, for two astrocytes. When analyzing the data, we can observe an average of a +/- 0.03 units difference between the manual analysis through MiniAnalysis and automated analysis through M.A.R.S. The n value next to the cell number is the number of ROIs in that astrocyte.

plitude, duration, and frequencies were compared between MiniAnalysis and M.A.R.S., we observed a small variation of +/- 0.03 units. This suggests that M.A.R.S. is comparable to measurements obtained through MiniAnalysis.

Several drugs have been used in research to emulate the function of neurotransmitters in astrocytic calcium signal modulation.<sup>11</sup> Drugs can alter calcium biology in different ways by modifying frequency, amplitude, and duration in different regions of the brain. Mitochondrial calcium signals in striatal astrocytes exposed to three different drugs, glutamate, quinpirole, and SKF, were measured with M.A.R.S.

Despite the large changes in amplitude, duration, and frequency of induced activity by drugs in astrocyte calcium, the extent of these changes was maintained from MiniAnalysis and M.A.R.S. As illustrated in **Figure 5**, there is a small variation of +/- 0.05 units between values obtained through manual and automated analysis. Combined, the effectiveness of analysis of the kinetics of both spontaneous and drug-induced events makes this custom-made MATLAB-based code

an important and innovative analysis tool for the study of astrocytic calcium signaling. Furthermore, an important and original feature is the generation of spatiotemporal maps to detect any patterns of both intensity and location of calcium events.

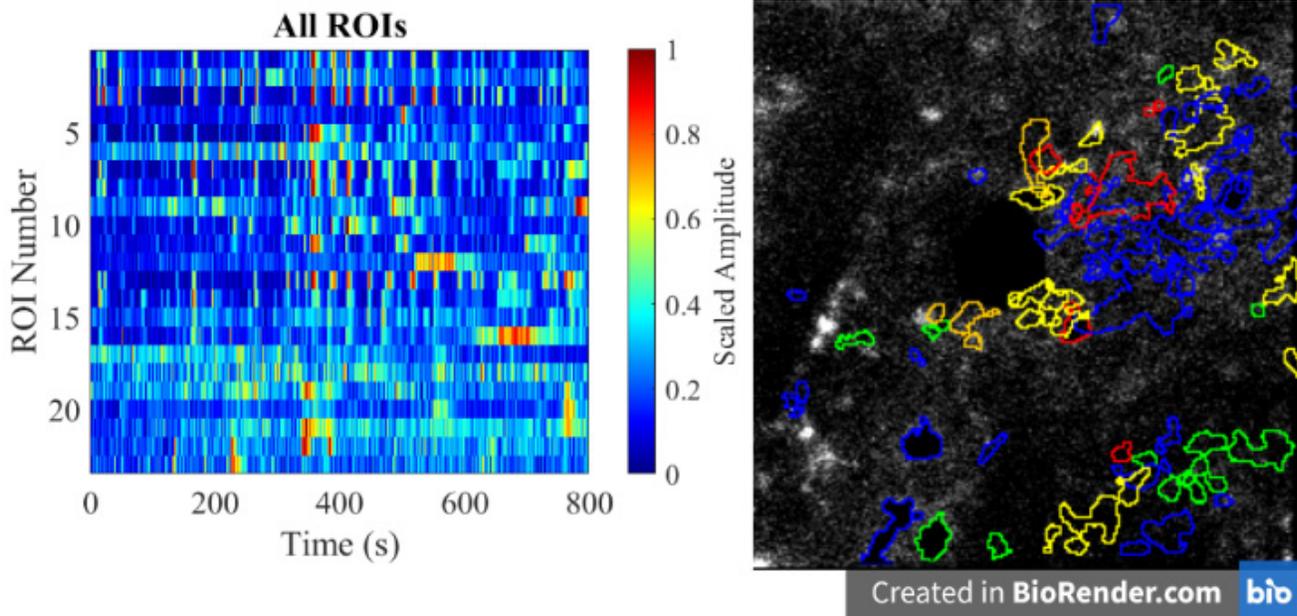
### *Semi-automated Generation of Spatiotemporal Maps using M.A.R.S*

The shape and surrounding of each individual astrocyte help determine its function. In some brain regions, astrocytes are surrounded by dendrites and not neuronal cell bodies, while in others they are surrounded by neuronal cell bodies. Due to this, the function of astrocytes may vary. At the same time, within one

		MiniAnalysis			M.A.R.S.		
		Amplitude	Duration	Frequency	Amplitude	Duration	Frequency
Cell 1 $n=26$	Soma	1.11	4.34	1.93	1.11	4.32	1.93
	Large Territory	0.67	3.29	1.60	0.65	3.29	1.60
	Small Territory	0.73	3.75	1.50	0.71	3.77	1.58
Cell 2 $n=9$	Soma	0.30	2.56	2.14	0.30	2.56	2.14
	Large Territory	0.23	2.29	0.53	0.22	2.29	0.54
	Small Territory	0.33	1.75	0.75	0.33	1.76	0.76

**Figure 5.** Amplitude, duration, and frequency measurements of two different astrocytes obtained with MiniAnalysis and M.A.R.S.. Cell 1 had an  $n = 26$  meaning that 26 ROIs were detected for that astrocyte and Glutamate was used to induce activity. Cell 2 had a  $n$  of 9, meaning there were a total of nine ROIs in that astrocyte and the Quinpirole was used to induce activity.

astrocyte one may also observe differences in different locations throughout the morphology of the astrocyte. To assess the differences in function, we asked if M.A.R.S. could also generate spatiotemporal maps of where these calcium signals are in astrocytes. Because of the exquisite relationship of astrocytic synapses with neighboring neuron synapses, the spatial location of these astrocytic mitochondria likely plays a role in synaptic modulation. To detect any potential firing patterns within different regions of an astrocyte, we implemented the generation of spatiotemporal maps that compare signaling intensity. These maps plot ROIs side by side, display increasing brightness by a change in color, and location of that change in brightness by correlating it to the frame number where it occurs.



**Figure 6.** Automated generation of spatiotemporal maps with M.A.R.S. After kinetic measurements, M.A.R.S. takes all the ROIs of the astrocyte being analyzed and generates a heat map (Figure 6A), that illustrates the increasing fluorescence intensity as the color changes from blue to red. Figure 6B shows the spatiotemporal representation of the heat map.

**Figure 6A** illustrates M.A.R.S. automated generation of heat maps, and the localization of the color-coded increasing intensities on the morphology of the astrocyte on **Figure 6B**. The tallest peaks plotted on the baseline corrected  $dF/F$  of each ROI of the astrocyte from which the map was generated, correspond to those red areas shown in **Figure 6**, both A and B. The correlation between the graphs and the maps further strengthens the claim that M.A.R.S. serves as an accurate and reliable method for mitochondrial astrocytic calcium analysis.

## CONCLUSION

Astrocyte calcium signals are thought to play important roles in behavior, as well as pathology of neurological diseases. Mitochondrial calcium signals in astrocytes have emerged as an important facet of the whole repertoire of calcium signals in astrocytes. Therefore, the accurate measurement of the kinetics of said calcium signals is of utmost importance.

Most existing programs for analysis of  $Ca^{2+}$  signals kinetics in astrocytes require manual intervention and are therefore time-consuming and inefficient. In this study we developed and tested our MATLAB-based program, M.A.R.S., that enables automated detection and analysis of astrocytic calcium signals. In addition, we described a semi-automated method in which M.A.R.S. can be used to develop spatiotemporal maps of spontaneous or drug induced mitochondrial calcium signals. Combined with the automated features and the astrocytic spatiotemporal map generation, M.A.R.S. is an accurate, relevant, and complete method for analysis and will likely impact the way we study and understand astrocytic biology.

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## **MARAY V. VALLE '22**

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# Prenatal Alcohol Exposure and Genetic Sex Impact Nfia Expression in the Developing Cortex

*By Aubrie Miller '22*

*Photo by Louis Reed on Unsplash.*

## INTRODUCTION

Prenatal alcohol exposure (PAE) can occur during pregnancy and result in fetal alcohol spectrum disorders (FASDs). PAE can cause physical abnormalities, including alterations to facial structure, growth deficits, and neurodevelopmental deficits caused by abnormal brain growth and development.<sup>1,2</sup> PAE is the leading non-genomic cause of learning disabilities.<sup>3</sup> Alcohol exposure during pregnancy and PAE can cause lifelong stigma for both the birth mother and the affected individual. This stigma contributes to additional hardships for individuals with FASDs including aggravating behavioral differences, mental illnesses, and substance use disorders as well as increasing barriers to achieving independence and life goals.<sup>4</sup> Women who consume alcohol during pregnancy are often labeled as bad mothers or child abusers, and therefore deserving of harsh judgment.<sup>5</sup> The stigma around alcohol exposure during pregnancy, combined with societal judgment, can make women reluctant to disclose alcohol use during pregnancy to their healthcare provider.<sup>6</sup>

Common binge drinking behaviors among men and women of childbearing age and lack of family planning combined with stigma against alcohol exposure during pregnancy means there is a low chance of eradicating PAE. Therefore, it is important to reduce harm both through social policies and by understanding the effects of PAE on brain development so that targets for intervention can be identified and the impact of PAE can be ameliorated.

Here, we investigated a potential mechanism of PAE's impact on brain development. Previously,

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we showed that PAE causes the premature maturation of neural stem cells (NSCs).<sup>7</sup> In the developing brain, NSCs proliferate to make additional NSCs (self-renewal) and differentiate to produce the cellular complement of the brain. NSC differentiation occurs sequentially. First, neurogenesis produces neurons. Then, astrogliogenesis produces astrocytes that maintain the chemical environment for neurons. Finally, oligodendrogenesis produces oligodendrocytes which create myelin around axons to provide electrical insulation. For astrogliogenesis, increased expression of transcription factor Nuclear Factor-1 A (NFIA) is necessary and sufficient to induce astrocyte production.<sup>8</sup> We hypothesized that PAE increases NFIA expression in NSCs, thereby inducing premature maturation through the premature activation of astrogliogenesis.

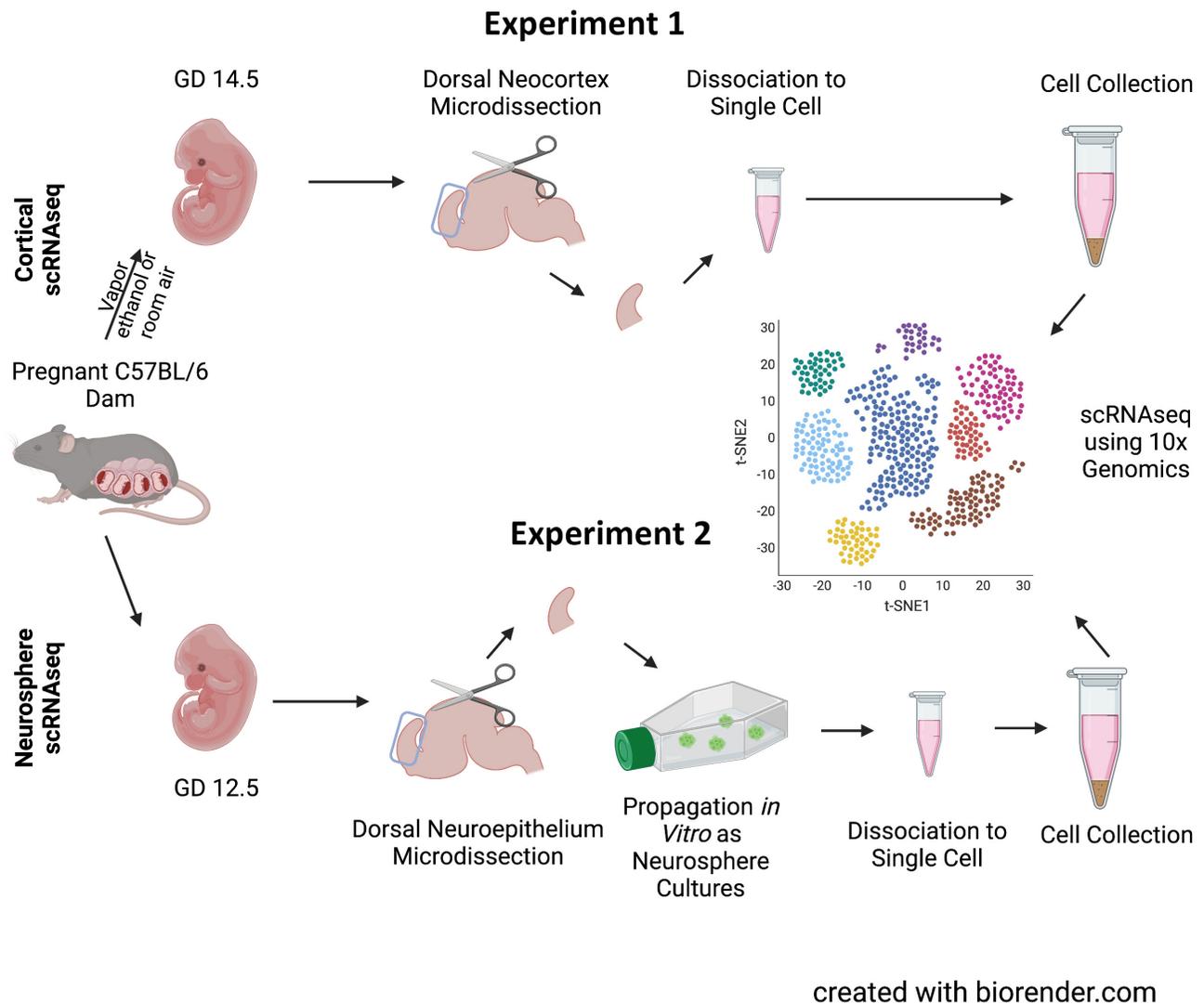
To determine if PAE results in premature activation of astrogliogenesis in NSCs, we examined if PAE increased the expression of *Nfia* mRNA during neurogenesis in the cerebral cortex using single-cell RNA sequencing (scRNAseq). scRNAseq allows analysis of transcriptional profiles within individual cells.<sup>9,10</sup> We performed scRNAseq on two mouse models of cerebral cortical NSC development: 1) in cortical tissue on gestational day (GD) 14.5 following exposure to ethanol vapor or room air on GD 12.5; 2) in neurosphere cultures grown from GD 12.5 mouse NSCs that were not exposed (naïve) to ethanol. Experiment 1 showed the impact of ethanol exposure on NSCs and their subsequent cycles of neurogenesis. Experiment 2 allowed us to compare the effects of ethanol from experiment 1 to isolated GD 12.5 NSCs grown as neurosphere cultures. For both of these models, cells from male and female fetuses were included, allowing for the assessment of impact of genetic sex on *Nfia* expression. GD 12.5–14.5 is the time of cortical NSC proliferation and the beginning of neurogenesis.<sup>11</sup> We chose to use two different models to better grasp the impact of ethanol exposure over time and examined cells at the age of exposure as well as the cells that differentiated following exposure. Furthermore, comparing these models provides insight on how the heterogeneity of the developing cortex may modulate the impact of PAE on NSCs.

We found that both genetic sex and ethanol ex-

posure affect *Nfia* expression. In GD 14.5 cortical cells, *Nfia* expression was higher in female stem cells, but in GD 12.5 neurosphere cultures, male NSCs have higher *Nfia* expression, suggesting that male NSCs may have higher levels of *Nfia* before female NSCs. In addition, in the GD 14.5 developing cortex, PAE increased *Nfia* expression in stem cells and early developing neurons.

Targeted interventions to restore normal *Nfia* expression may slow premature maturation of NSCs

and reduce the detrimental changes to brain development following PAE. But these interventions may need to be genetic sex-specific, which impacts the timeline of *Nfia* expression. These interventions may be one effective way to reduce the harm of PAE and improve long-term quality of life for individuals with FASDs.



**Figure 1.** Paradigm for sample collection. Cortical scRNAseq (experiment 1, top) used tissues from gestational day 14.5 (GD 14.5) fetuses following the exposure to ethanol or room air vapor on GD 12.5. Following microdissection of the dorsal neocortex, tissue was dissociated into single cells which was then further analyzed using the 10x Genomics platform. Neurosphere scRNAseq (experiment 2, bottom) used cortical tissues from GD 12.5 fetuses which underwent the same dissection; however, they were then propagated as neurosphere cultures then analyzed by scRNAseq. Created with biorender.com using a premium license made available through the Texas A&M Institute for Genome Sciences and Society.

## METHODS

### Cortical scRNAseq

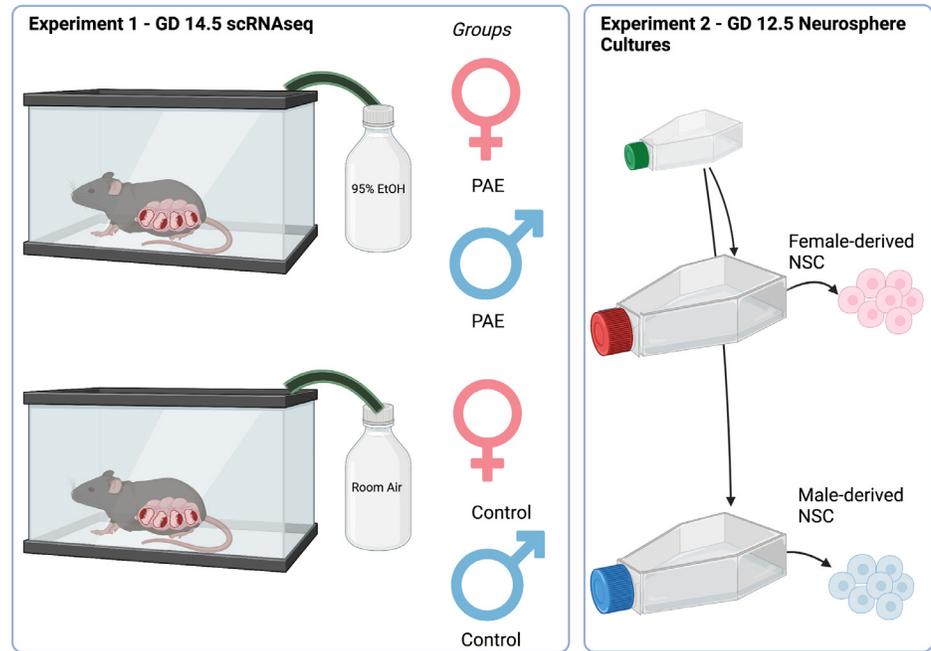
For experiment 1, pregnant C57BL/6 mouse dams were exposed to vaporized 95% ethanol or room air on GD 12.5 (**Figure 1**). Ethanol exposure resulted in blood ethanol concentrations ranging from 210–260 mg/dL (equivalent to blood alcohol concentrations, or BACs, of 0.21–0.26%). Two days following exposure, on GD 14.5, the fetuses were removed and underwent microdissections to obtain the dorsal neocortex. The collected tissues were then broken down into single cell suspensions by manual trituration and enzymatic disaggregation. Cells were collected by centrifugation, briefly fixed in methanol, and underwent scRNAseq using the 10x Genomics pipeline (**Figure 2**). Initial characterization of these data has been previously published.<sup>12</sup>

### Neurosphere scRNAseq

In experiment 2, similar microdissections occurred as in experiment 1. However, dorsal neuroepithelium was collected from GD 12.5 fetuses. Tissues were broken into single cell suspensions and propagated in vitro as neurosphere cultures in mitogenic media. These cells, following 5 days of growth in mitogenic media, were separated into single cell suspensions, briefly fixed with methanol, and underwent scRNAseq using the 10x Genomics pipeline. Initial characterization of these data has been published.<sup>13</sup>

### Segregation of Tissues by Genetic Sex

At the time of tissue collection for both exper-

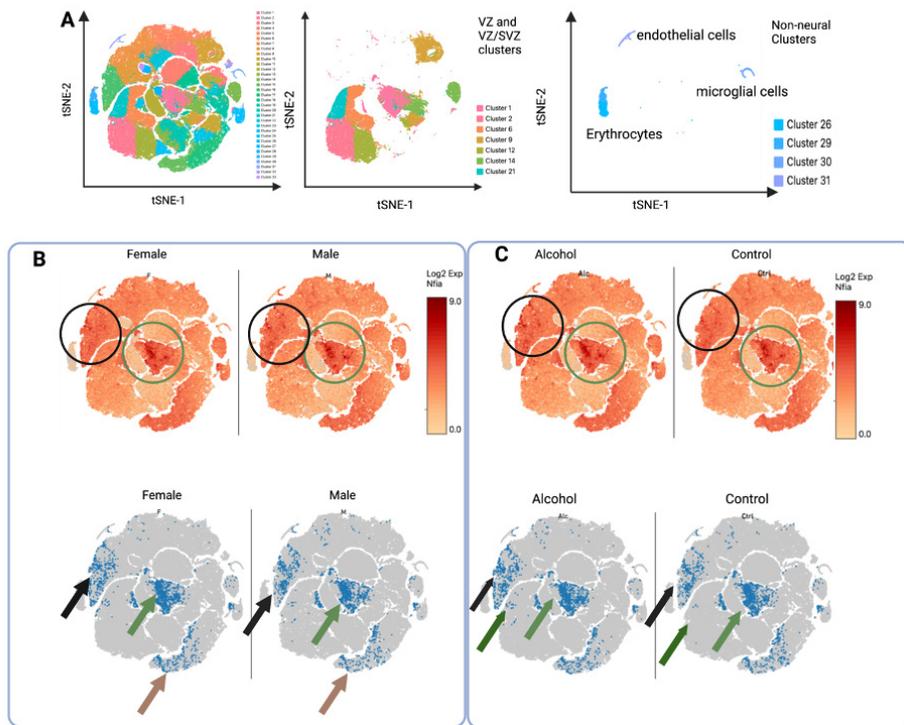


**Figure 2.** Exposure paradigm. In experiment 1, pregnant dams were exposed to either 95% ethanol or room air via a vapor chamber on GD 12.5. Subsequently, on GD 14.5, tissue from the exposed fetuses were then segregated by genetic sex. In experiment 2, GD 12.5 NSCs were propagated as sex-segregated neurosphere cultures in mitogenic media. Created with biorender.com using a premium license made available through the Texas A&M Institute for Genome Sciences and Society.

iments, additional fetal tissue was taken to determine fetal sex. DNA was extracted from these tissues by alkaline lysis. This DNA suspension was then neutralized, diluted, and underwent quantitative real-time polymerase chain reaction (qPCR) for repetitive sequences present on the X and Y chromosomes.

## RESULTS

Data obtained from scRNAseq is complex and multi-dimensional. These data can be visualized in low-dimensional space by using the t-distributed Stochastic Neighbor Embedding (t-SNE) algorithm to construct t-SNE plots. In these plots, each data point represents a single cell, and cells are clustered by similarity and separated from dissimilar cells. For both the cortical scRNAseq (**Figure 3A**) and the neurosphere scRNAseq (**Figure 4A**), a number of discrete cellular clusters were identified by the t-SNE algorithm, although 33 clusters in total were identified for



**Figure 3.** T-SNE plots of experiment 1, cortical scRNAseq. **A.) LEFT:** color coded t-SNE plot of the 33 identified cell clusters in the cortex. **MIDDLE:** clusters of the VZ and VZ/SVZ regions. **RIGHT:** non-neural clusters. **B. and C.)** *Nfia* expression in cortices stratified by genetic sex, regardless of treatment (B) or exposure, regardless of genetic sex (C). **TOP:** *Nfia* expression in all cells as indicated by color coding with log2 scale of expression in which higher expression is colored red while low rates of expression are colored yellow. **BOTTOM:** the bottom 10% of *Nfia* expression was excluded to show a clear picture of sex differences in number of cells with *Nfia* expression. **B.)** The green circle and arrow highlight cell populations with higher *Nfia* expression in female-derived cells while the black circle and arrow and brown arrow highlight cell populations with decreased *Nfia* expression in female-derived cells. The green region contains NSCs in an VZ cluster, black region contains neurons of lateral layer V/VI neurons, and the brown region contains neurons of rostral layer V/VI. **C.)** The black circle and arrow highlights layer V/VI cells with greater *Nfia* expression in the alcohol group and the green circle and arrows highlight VZ cells with higher expression in the alcohol group.

the cortical scRNAseq and 10 were identified for the neurosphere cultures.

### Cortical scRNAseq

In previous work,<sup>14</sup> cellular clusters were identified based on gene expression to correspond to cellular populations in the developing cortex. Here we focused the regions of the developing cortex that contain NSCs: the ventricular zone (VZ), which contains the earliest, most multipotent neural stem cells and the sub-

ventricular zone (SVZ), which contains another population of stem cells that divide more rapidly to expand the cortex (**Figure 3A**). In cell clusters that contained cells identified as belonging to the VZ, SVZ, or both (VZ/SVZ), we first compared *Nfia* expression by genetic sex (**Figure 3B**). We found in the central VZ cluster that female-derived cells have higher expression of *Nfia* than male-derived cells. We further examined the number of cells that have *Nfia* expression, excluding those cells in the bottom tenth percentile of expression. More female-derived cells express *Nfia* in this VZ cell cluster than male-derived cells. While examining the *Nfia* expression in the developing cortex, we noticed that sex difference in *Nfia* expression seen in NSCs was not similarly seen across all cell types in the GD 14.5 cortex. Contrastingly, clusters that correspond to early neurons (layers V/VI) have higher *Nfia* expression in male-derived cells compared to female-derived cells. We then compared *Nfia* in the cortical cell cluster stratified by exposure (**Figure**

**3C**). In this comparison, we found that ethanol exposure increased *Nfia* expression in the stem cells of the central VZ cluster and in the early layer V/VI neurons of layer V/VI. These data suggest that sex differences in *Nfia* expression in the developing cortex are cell-type specific while ethanol may similarly regulate *Nfia* across cell types.

For the construction of t-SNE plots for the neurosphere scRNAseq, when the data from the male-

and female-derived neurospheres was combined, the cell clusters largely segregated by sex.<sup>15</sup> Therefore, we constructed two independent t-SNE plots, one for each sex, and used gene expression data to determine closely related clusters across plots (**Figure 4A**).

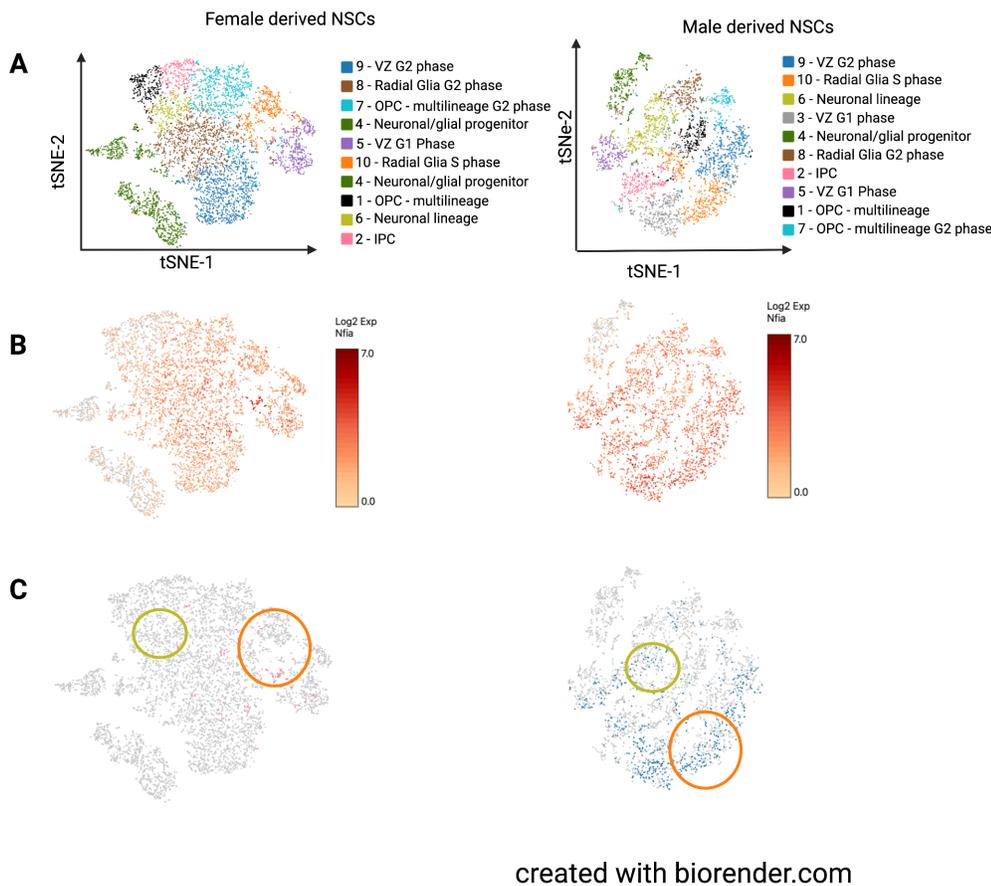
Corresponding clusters are color-coded the same even though they may not be in the same location. For the neurospheres, almost all clusters had markers for neural stem/progenitor cells with the exception of cluster 4, which had higher expression of differentiation lineage markers. We found that *Nfia* is expressed similarly across NSC populations in both male and female neurospheres. However, when we

examined individual cells, only a few female cells had considerable *Nfia* expression (>10<sup>th</sup> percentile of expression seen in both male and female neurospheres). A small group of cells expressed *Nfia* in female-derived neurospheres but this group had widespread expression in male-derived neurospheres. Other cell populations had almost no cells with *Nfia* expression in the females, while the males had widespread expression.

## CONCLUSION

Here, we showed that neural stem cells in the developing cortex are not a homogenous population. Cortical scRNAseq defined 11 VZ cell clusters while

the neurosphere scRNAseq had 10 clusters. We found sex-differences in the expression of *Nfia* in NSCs, with females showing higher expression at GD 14.5 in the central VZ NSC cluster and males showing higher expression in the GD 12.5 NSC cultures. Ethanol generally increased *Nfia* expression, although this effect was NSC subpopulation specific, as we did not see similar increases in expression across all the VZ/SVZ clusters. We found that *Nfia* expression level was widespread in NSCs but also varied by NSC subpopulation. Surprisingly, even in NSC clusters with



**Figure 4.** Experiment 2, neurosphere scRNAseq. **A.)** Both-t-SNE plots of cell clusters in male- and female-derived neurosphere clusters. Similar clusters were present in each plot with the exclusion of cluster 3 from the female plot. **B.)** *Nfia* expression in all cells as indicated by color coding with log<sub>2</sub> scale of expression in which higher expression is colored red while low rates of expression are colored yellow. **C.)** Cells expressing *Nfia*, with cells in the bottom 10% of *Nfia* expression excluded. Orange circles highlight cluster 10 which contained radial glia-like neural progenitors in S phase. Yellow circles highlight cluster 6 which contained the neuronal lineage marker-expressing progenitor cells.

## TARGETED INTERVENTIONS TO NSCs HAVE THE POTENTIAL TO IMPROVE NEURODEVELOPMENT, AND THEREFORE QUALITY OF LIFE FOR INDIVIDUALS WITH FASDS

high *Nfia* expression, there was not a corresponding expression of astrocytic cell markers. These data indicate that *Nfia* is expressed in NSCs prior to astrogliogenesis and may define NSC subpopulations.

These data indicate that PAE can induce *Nfia* expression in NSCs and this increased expression may be targetable for intervention. However, any intervention will likely need to be tailored to each genetic sex, as we found sex differences in the expression timeline of *Nfia*. By decreasing *Nfia* expression following PAE, we may encourage NSC self-renewal, reducing harm to the developing cortex caused by PAE. Targeted interventions to NSCs have the potential to improve neurodevelopment, and therefore quality of life for individuals with FASDs. Further investigation is needed to discover the best methods of intervention of NSCs and other targets that could work alongside *Nfia*-targeted therapeutics to reduce harm following PAE and ameliorate more negative outcomes associated with FASDs.

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### **AUBRIE MILLER '22**

Aubrie Miller '22 is a biochemistry and genetics major from Fort Worth, Texas. Aubrie is passionate about women's health and prenatal care. She hopes to go to Medical School to pursue a career in meeting women's needs.

# Controversial Saints: A Study of How Popular Culture Can Radicalize Religious Icons

*By Alexandria Babineaux '22*



## INTRODUCTION

“Queer,” “lesbian,” and “gender nonconforming” are not terms one often relates to the Catholic Saint Joan of Arc. Depictions of Joan of Arc in media have transformed dramatically over time, often being impacted by popular culture, and many modern interpretations of her include themes of queer identities. As an artist and a member of the LGBT community, I became fascinated with the dichotomy between the identities of Joan of Arc and was inspired to create my own take on this “controversial” saint.

While intrigued by figures in the Bible and motifs in religious art, Christianity and Catholicism’s stance towards LGBT people like myself began to push me away from religion. However, I have never been able to shake my love of religious iconography. Through my studies, I discovered that the worlds of Christianity and the LGBT community often collide in the realm of art. This realization, along with my own personal history, led me to further study the intersectionality between the LGBT community and the Christian and Catholic religions with an emphasis on depictions in digital media. This research has resulted in the creation of a piece of art centered on Joan of Arc and her controversial stance as both a saint and gay icon.

## BACKGROUND

Born in 1412 during the Hundred Years War of France versus Britain, teenage Joan of Arc dressed as a man and fought for France under the guidance of God and the Archangel Michael.<sup>1</sup> She was eventually captured by the British and burned at the stake for witchcraft and heresy at only 19 years old. In 1920, 500 years after her death, Joan of Arc was anointed as the patron saint of France and assumed the identity most know her by today.<sup>2</sup>

The subject of Joan of Arc’s identity has been a turbulent one, both during her life and after death. In modern times she is known not only as “The Maid of Orleans” and the patron saint of France, but also as a feminist symbol, a queer icon, and even as a character in multiple massive media franchises. These many

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faces of Joan of Arc are often contradictory, especially her stance as both a Catholic saint and a “queer icon.” However, her status as a “gay saint” and Catholic saint shows that there is a desire for representation of individuals who exist in the intersection of these communities.

My interest in the transformation of saints into gay icons was sparked in an art history class with Dr. Stoenescu of Texas A&M University’s Visualization department. One of the subjects was Saint Sebastian who, like Joan of Arc, was transformed from a Catholic saint into a gay icon. Saint Sebastian’s transformation came about via influences of the Renaissance art world.<sup>3</sup> Artists of the Renaissance were in pursuit of creating the perfect male nude as a showcase of their artistic skill. Saint Sebastian became the subject of these paintings, and as time went on, depictions of him became less religious and more erotic. During the height of the Renaissance, creating a painting of Saint Sebastian or owning paintings of him symbolized male homosexuality.<sup>4</sup> For example, Leonardo Da Vinci, who was a very outspoken gay man, owned eight paintings of Saint Sebastian.

In modern times, people often assume that queer people did not exist in the past or were not open about their identities, but the opposite is true. There is a long history of using art as a means for queer people to express themselves that continues to this day.

Of the many who can be classified as a “gay saint,” Joan of Arc stood out to me most as I related to her struggles of being a woman persecuted for how she presents herself. I was also inspired by her steadfast dedication to her beliefs, even in the face of death.

Joan of Arc's journey from peasant girl to nationalist icon, then to queer icon and subject in modern media is lengthy and complicated. It can, however, be traced to three major events that sparked a shift in the perception of Joan of Arc in media.

The first shift is attributed to the Action Française movement, which was an anti-republican and pro-monarchist movement active in France during the early 1900s.<sup>5</sup> Its members found themselves in a propaganda war with the pro-republican movement. Their opponents had claimed many popular symbols such as The Marianne, a female personification of the French spirit. To compete with their rivals, Action Française made Joan of Arc the figurehead of their campaigns. During this period of political turmoil, Joan of Arc was venerated as a saint and became the official patron saint of France.<sup>6</sup> The overlap of these events led to the overwhelming success of Joan of Arc's status as a symbol of French Nationalism.

The next shift in Joan of Arc's identity came as a result of her renewed popularity with the general public. Many new creative works were sparked after Joan of Arc obtained sainthood, one of the most influential being Vita Sackville-West's biography of her published in 1936.<sup>7</sup> This novel is widely attributed as the first instance of speculation on Joan of Arc's gender and sexual identity. Vita Sackville-West was a popular English poet and novelist with dozens of publications and several awards. Sackville-West also led an eventful love life, with both male and female lovers. She would often cross dress as a man when out in public with her female lovers to avoid hostility and discrimination. These details of her personal life were published after her death by her son in "Portrait of a Marriage" in 1973.<sup>8</sup> In her essay "Cross Dressing for (Imaginary) Battle," author Kathryn Z. Sproles states that Sackville-West may have projected her own identity and struggles onto Joan of Arc in her novel, insinuating that

Joan of Arc was also a cross dressing queer woman.<sup>9</sup>

Since the publication of this novel, there has been much speculation about the "true" identity of Joan of Arc. Alongside the theory that she was a cross dressing lesbian, others believe she was asexual, a transgender man, or even genetically male but suffering from a hormonal illness.<sup>10</sup> A peasant girl alive in the 1400s may have identified as any of the above but would not have had the language to say so in her trials. Alternatively, she could have simply been dressing as God told her, as Joan states very clearly during her trials. Whatever her "true" identity may be, in modern times, Joan of Arc has become a symbol for people who face similar persecution, and many find solace and inspiration in her perseverance.

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The last shift in the perception of Joan of Arc can be attributed to a Japanese media franchise. To fully grasp this shift, it is imperative to search "Joan of Arc" and then "Jeanne d'Arc" in an image search engine. "Joan of Arc" results in historical paintings and images from modern movies. "Jeanne d'Arc" however, shows a

woman with a long blonde braid, an ornate headpiece, and purple armor. This is Joan of Arc, renamed Jeanne d'Arc, as she is depicted in the Japanese *Fate Apocrypha* and *Fate Grand Order* franchises. This version of Joan of Arc made her debut in the first volume of the novel *Fate Apocrypha* in 2012.<sup>11</sup> The series was a massive success, generating an animated TV show and a comic adaptation of the story. When the mobile game *Fate Grand Order* was released in 2015, Jeanne d'Arc became the subject of its first major story section and a poster girl for the series.<sup>12</sup> The personality of *Fate's* version of Jeanne may not be historically accurate, but that has done nothing to discourage fans from loving her, as she is massively popular across the world. The Japanese image hosting website Pixiv has over 22,000 images tagged "Jeanne d'Arc," and the fanfiction website Archive of Our Own, or AO3, has over 600

entries for her name.<sup>13</sup> Many of these fan works feature queer and gender nonconforming depictions. Some of the most popular include hypothetical pairings, such as a romantic relationship between Jeanne d'Arc and Marie Antoinette. The creation of these fan works and their popularity shows how well accepted Joan of Arc's status as a queer icon has become. Fate's version of Joan of Arc is so popular that it overwhelms search engine results for her French name, and often appears before historically accurate depictions of Joan of Arc in search listings. Her existence in this series has created a gateway for non-Christian individuals to discover her and become invested in the legacy of Joan of Arc. Through the internet, social media, and popular culture, a simple peasant girl who lived 500 years ago has become an international media sensation with millions of fans worldwide.

## CREATIVE ARTIFACT

For my artifact, I wanted to create art that pays homage to art history and acknowledges the importance of modern media on the identity of Joan of Arc. I created a digital painting in the program Clip Studio Paint that combines digital rendering and traditional painting methods and compositions. I then animated it in the program Live2D. The choice of digital mediums is in reference to how much digital media and internet culture contributes to the discussion of homophobia and LGBT acceptance. The result is a dynamic digital painting that critiques Christian churches' homophobic rhetoric and aims to raise awareness of the negative effects it has on LGBT people.

Through my creative process, I was drawn to the motif of fire and the subject of identity and agency. After reading the transcripts of Joan of Arc's trial, I was struck by how proud and confident she was until the moment of her death. Joan of Arc may have been the victim of cruelty, but she always retained her own dignity and agency. I was also captivated by the idea of fire as a symbol of strength and rebirth, and of Joan's tragic death.

I found myself unsure of how to visualize my intentions and research motivations for some time. As

much as I love modern representations of a butch lesbian Joan of Arc, or depictions of her wearing a binder as a symbol for transgender people, those images were not aligned with my intentions. I did not want to make a statement on what I personally think the true identity of Joan of Arc was, because I believe that each person's unique interpretation of her is valid. Instead, I wanted to focus on her martyrdom and how I could use that to convey a message.

I went through many variations and my strongest ones always containing the motif of fire and Joan's three heavenly messengers. Joan speaks of her visits from Saints Catherine and Margaret, as well as the Archangel Michael positively. Her enduring faith in their words led her to glory, but also to her death. I wanted to take this event that is often hailed as a great act of God and the epitome of a "good Christian" and subvert it.

My final composition depicts Joan of Arc flanked by her three heavenly messengers. Joan holds a flaming sword near her neck, with the hands of the two female saints supporting it upwards. The angel Michael stands behind the three and is represented according to descriptions of Seraphim in the Bible. As the animation plays, Joan looks between the viewer and her sword as the saints and the angel force her to move the blade towards her neck. Fire roars around them, and Michael's wings move to surround Joan and the two saints. The animation loops continuously (**Figure 1**).

## DISCUSSION

Through my research into the history of Joan of Arc, I found myself contemplating her unique existence

**I WAS STRUCK BY HOW  
PROUD AND CONFIDENT  
SHE WAS UNTIL THE  
MOMENT OF HER  
DEATH.**



**Figure 1.** *Controversial Saints: Joan of Arc. Animated digital painting. Alexandria Babineaux.*

as both a gay saint and a Catholic saint. The intersection between these two communities is often ignored but does exist. According to a 2020 survey conducted by The Williams Institute UCLA School of Law, nearly half of LGBT adults are religious and approximately 25% of them are Catholic.<sup>14</sup> This is no small denomination; this is a large minority group that is often overlooked if not intentionally ignored.

As someone who exists in the intersection of these two groups, I often find myself at odds with both. I have not felt acceptance from the Catholic Church or other Christian churches due to their belief that homosexuality is a sin. Many LGBT people have religious trauma from abuse and discrimination and understandably, they shy away from subject of Christianity as a whole. However, the people who do exist in this intersection are drowned out by the fighting between the two.

These struggles are what led to my research question and the concept behind my creative artifact. I wanted to create a piece of art that serves as a springboard for discussion of the effects growing up in



Scan the QR code to watch the Figure 1 animation, or visit <https://tx.ag/522Fig1>.

religious communities can have on LGBT people, as well as to bring awareness to the discrimination LGBT people face by these same religious groups. To convey this message, I chose to subvert Joan of Arc's great act of obedience to the word of God. I transformed it into a metaphor for how Christian churches often use the Bible and word of God as justification for their hatred and bigotry. My choice to have Joan of Arc as the subject

of this act is to literally turn Saint Joan of Arc into a sinner. By depicting a beloved saint as a sinner and the victim of God's wrath, I aim to draw attention to our common humanity and the idea that no one deserves the cruelty and abuse that is often perpetuated in the name of God.

## CONCLUSION

While conducting this research, I could not help but think about how much good it would do if the Catholic Church would accept Joan of Arc's identity as a "gay saint." It would bring solace to ostracized queer Catholics and Christians and would help undermine the Catholic Church's hatred of specific groups. Instead, the Vatican authority's official dogma continues to insist that all members of the LGBT community, regardless of gender identity or sexuality, are sinners.<sup>15</sup>

The subject of historical accuracy versus modern interpretations often comes up in discussions of Joan of Arc. But in terms of her status as a modern saint, does historical accuracy even matter? Catholic saints exist to be beacons of hope and inspiration for the masses. Many saints have dedications that have nothing to do with their original lives. If a saint brings solace and hope to any group of people, that should be accepted and celebrated. I hope that the discussion created by this creative artifact can help foster a better understanding between these two groups and promote acceptance regardless of religion or personal identity.

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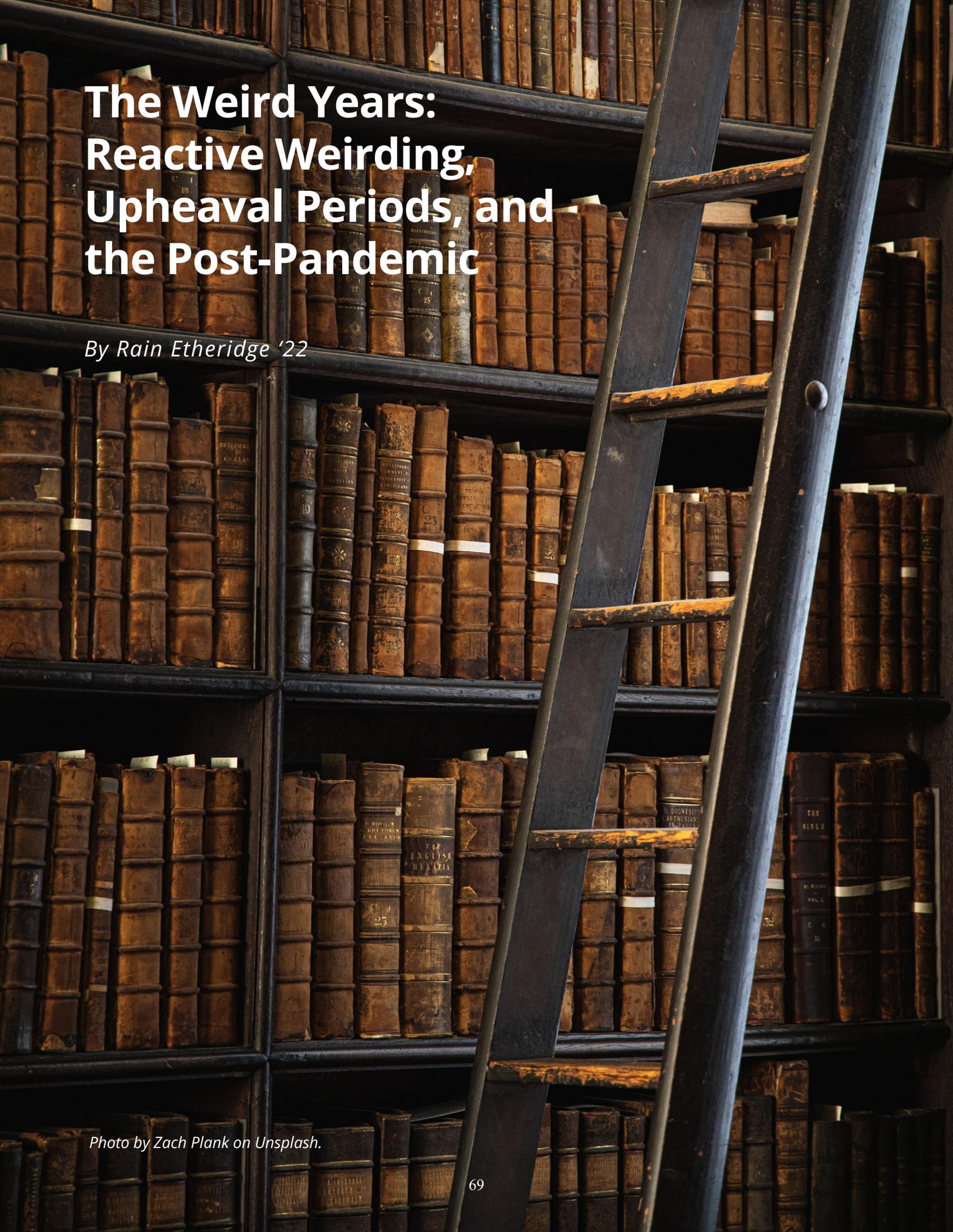
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## **ALEXANDRIA BABINEAUX '22**

Alexandria Babineaux '22 is a visualization major from College Station, Texas. Her research is motivated by her love of art history and desire to explore modern digital artistic technology. She participated in the Aggie Creative Collective to produce a creative artifact to accompany her research. After graduation, Alexandria plans on pursuing a master's degree in fine art and a career in research and artistic education at the college level.



# The Weird Years: Reactive Weirding, Upheaval Periods, and the Post-Pandemic

*By Rain Etheridge '22*

*Photo by Zach Plank on Unsplash.*

*“Yet, I also began to have the sense... that around the world enclaves that never knew one another—writers who could not have read each other... had stared up at the same shared unfamiliar constellations in the night sky, heard the same unearthly music: a gorgeous choir of unique yet interlocking imaginations and visions and phantoms. At such times, you wonder as both a writer and an editor if you are creating narrative or merely serving as a conduit for what was already there.”*

-Jeff Vandermeer, *The Weirid*

## MOTIVATION

When examining the fringes of genre literature, a set of terms keep appearing: “bizarre,” “uncanny,” “surreal,” and “unnatural.” Taken together, these concepts can be called the Weirid—the domain of unnamed monsters, cryptic revenants, and horrifying mutations.

The Weirid as a discipline is widely underestimated. It retains a stigma in the publishing world, its role in pop culture is heavily understated, and it remains chronically understudied in academia, despite some sporadic interest in the last two decades.<sup>1</sup> But what if the Weirid represents something more fundamental about literature? What if there is something embedded in history, politics, even ecology, that beckons the Weirid out of the background like a chthonic siren? And how, if at all, is this strange force shaping new literature as the pandemic fades?

Before we can answer these questions, allow us to consider two distinct (but non-exclusive) possible definitions of what we might mean by the Weirid:

1. A subgenre of popular fiction combining elements of fantasy, horror, pulp, and science fiction that is primarily distinguished by a distorted, transformed, or corrupted perception of reality. Stock characters and tropes from the parent genres often appear but are significantly altered to the point of strangeness.<sup>2</sup>
2. A mode of storytelling, represented occasionally in most, if not all, categories of genre and literary fiction. It is characterized by the

interrogation and subversion of established reality narratives. By calling into question said narratives, the Weirid mode typically seeks to convey the points of view of those individuals and ideas that lie outside the bounds of polite, mainstream culture.<sup>3</sup>

Definition one treats the Weirid as most do. It examines only the literary tradition of popular horror that evolved with those such as Edgar Allan Poe, Mary Shelly, and H. P. Lovecraft from the Gothic. It is specific, unassuming, and precise. It also misses much that is fundamental about what makes us as a culture return perennially to the Weirid. For that, we must look to definition two: the Weirid as a mode.

Mode refers to a collection of methods, moods, and impetuses. This is different from genre as we typically discuss it, as genres imply a common history and lineage. They are primarily *ad hoc* categories used to manage audience expectations and to study the evolution of literary ideas. If we think of genres as trees in a forest, each with their branches of influence, then modes are the terrain, or the geology. They support and underlie many genres simultaneously. For example, fantasy proper utilizes what might be called the Fantastic mode, which deals with playfulness and self-reflectiveness of language. However, this mode is by no means exclusive to fantasy, also appearing in genres such as science fiction and horror. Unlike genres, modes are unbound by history and time—they are different ways of answering the question: “Why tell these stories at all?”<sup>4</sup>

When viewed as a mode, the Weirid appears in unexpected places outside of the genre that bears its name. Magical realism, a subgenre developed in Latin America in the early twentieth century and later iterated upon globally, draws from the Weirid as much or more than Lovecraftian horror does. It blends extraordinary and mundane elements in such a way that the two become inseparable, thus deconstructing reality narratives in the exact manner we described in definition two. Postmodernism, the primary advancement of Western postwar literature, was saturated with the Weirid in a similar way to magical realism. Authors sought to de-

construct the narratives of the past, and tore down reality itself to do so. Metamodernism, the recent evolution of postmodernism into the twenty-first century, pushed popular literature further into Weird territories. Modern science fiction is Weirder than ever. The list goes on.

Across history, the Weird appears in “clusters,” explosions of genre and subgenre that become popular in specific places at specific times. This phenomenon alone is an indication that something fundamental is at work in the various emergences of Weird fiction beyond genre trappings. Mode can begin to explain this clustering.

All Weird fiction, whatever the genre, is born of alienation, dislocation, depersonalization, depression, and/or delirium. The feeling, in whatever form, that something has changed, that while one was not looking, something impossible slipped in and replaced what one thought they knew to be real. Weird fiction in all its forms is dedicated to the expression and exploration of this sensation.<sup>5</sup>

What drives readers and authors to interrogate the often-uncomfortable nature of change? For that, we must look to cognitive linguistics.

Linguists Ostergaard and Bundgaard have proposed that new forms of literature emerge out of the contact between two forces: the texts that came before, and the “exigencies” of the current historical context.<sup>6</sup> Exigencies are forces external to the writing process, and they come in two forms, Constrictions and Affordances. Constrictions are things like scarcities, taboos, unknowns, and repression—they disincentivize certain topics from being emphasized. Affordances are the opposite: fads, abundances, new information—they encourage certain traits to be played up. The tension between positive and negative exigencies creates a set of incentives that are unique to that time and place. An

author responds to these incentives by iterating on the works that have come before her to create something new, and a novel form of literature is born. This process goes on to influence history, changing the exigencies further. Ostergaard and Bundgaard call this phenomenon the “Dual Feedback Loop,” and it helps to explain why Weird musings tend to cluster.<sup>7</sup>

The exigencies, or external circumstances, that produce Weird works are those which produce alienation. Exigencies arise when culture, politics, ecology, economics, and/or technology shift to the point that previous ways of life become unrecognizable. These disturbances may be the reason why the Weird seems to cluster so heavily—it is written in response to periods of extreme change—thus earning the term “reactive

weirding.” “Reactive weirding” is the property of literature to transform itself, to weird itself, as a reaction to systemic upheaval. These extreme shifts may be the commonality behind explosions of the Weird.

This link is made relevant by the current transformational moment. Since the beginning of the COVID-19 pandemic (and arguably before), most

aspects of American life and society are in flux. By understanding the response of historical societies to such changes, we may be able to predict the coming literary moment.

To investigate the potential association between change and the Weird, it will be necessary to review several Weird movements of the twentieth and twenty-first centuries.

## HISTORY

Two useful examples of Weird explosions are the development of magical realism in Latin America as a reaction to chronic instability, and the development

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of metamodernist maximalism in response to the rise of mass media.

While its precursor movements can be traced back to 1920s Germany, the subgenre known today as magical realism found its footing in Latin America beginning in 1955. Authors like Arturo Uslar-Pietri, Jorge Luis Borges, and Gabriel Garcia Marquez all pushed the form forward with their bold insistence on fantasy-as-fact, which would become the hallmark of the genre. Magical realism distinguished itself with its colorful and often near-psychedelic approach to reality, in which fantastic and impossible elements mingle seamlessly with real-life until the two are inseparable. This disregard for the “realistic” is a classic example of Weirding. By making reality strange and often incomprehensible, writers like Marquez were able to call attention more easily to aspects of reality they found mentionable.<sup>8</sup>

And what aspects were mentionable were, invariably, political. Latin America’s postcolonial relationship with Europe bred a kind of marginality—Latin America was often seen as being at the periphery of European culture. The violence wrought by colonialism had still not been adequately recognized, and the impacts of rapid decolonization had plunged many nations, such as Colombia, into cyclical periods of intense revolutionary and dictatorial violence. When Marquez was growing up, events such as the Thousand Days’ War and the banana massacres, bloody clashes over politics and labor, were intimately entwined with his childhood and family history. The influence on his work is far from subtle. Books such as *Autumn of the Patriarch* and *One Hundred Years of Solitude* openly comment on the upheaval and precarity of his time.<sup>9</sup> The violence and surreality of his work are reflections of the environment it was created in. It could be said that without the upheaval of the period, the subgenre of magical realism would have been unable to form the way it did. The weirding inherent in stories such as *A Very Old Man with Enormous Wings* allowed Marquez’s writing to speak so eloquently and succinctly to the attitudes of the time.

Marquez and his contemporaries inspired similar traits in authors overseas as magical realism spread

from Latin America. Jose Saramago began to use magical realism to describe pandemics, social unrest, and the evolution of religion in his home country of Portugal in the 1970s, ‘80s, and ‘90s. Saramago’s quietly dark writing evokes Marquez, but in a uniquely Portuguese context. His works reflect pointedly on the issues of his day and locale. Novels such as *Blindness* tackle the decay of authority in the late stages of capitalism, and the failure of Portuguese society to reckon with the massive changes of the late twentieth century.<sup>10</sup>

Concurrently, authors in the United States and elsewhere were exploring evolutions of postmodernism to achieve a similar effect with slightly different techniques.

While mass media had already been asserting an effect, the latter half of the twentieth century brought about a paradigm shift in the way Americans consumed and thought about media. The John F. Kennedy presidency and assassination, Vietnam, and Watergate were all culturally consequential events that were mediated primarily through the television screen. By the 1980s, TV had entrenched itself as an organ of power and ideology, embodied by Reagan, the former host of *General Electric Theater*. Soon after, the 24-hour news cycle would ascend, popularized by the OJ Simpson trial. In part, the societal changes brought about by television and the nascent World Wide Web instigated an evolution beyond postmodernism in the late 1980s, 1990s, and early 2000s.<sup>11</sup>

Pop culture was first made aware of this evolution in 1996, with the release of David Foster Wallace’s 1,000+ page novel *Infinite Jest*.<sup>12</sup> The book was a best-seller and received praise from critics for its originality, humor, and intellectual depth.

What often goes unmentioned is the degree to which Wallace’s distinctive style leans on the Weird. The world of *Infinite Jest* is subtly but pervasively magical realist. New England and Quebec have been reduced to a smoldering trash heap. Herds of giant hamsters and overgrown, malformed fetuses occasionally make an appearance. Absurdity of all kinds overflows from the most basic of human interactions, seeming to spill out of the social and into the actual.

And of course, the central plot device, the fictional film from which the title is taken, is a movie so entertaining that once one has seen it, they cannot resist watching it until they die.

Speculation on the motivations of Wallace's excursion into the Weird is largely unnecessary. The use of magical realist imagery, and more broadly the Weird tone, is repeatedly cast within the novel itself as a byproduct of the sensationalism and entertainment addiction that had rapidly overtaken America. To Wallace, television seems less an evil in and of itself and more so a symptom of some greater emptiness in America's center. It is this emptiness, this *alienation*, and the struggle to alleviate it, with which *Infinite Jest* is concerned.<sup>13</sup>

Other books utilizing similar techniques followed, and in some cases preceded *Infinite Jest*. Mark Z. Danielewski's linguistic puzzle box *House of Leaves*<sup>14</sup> and Reza Negarestani's work of fictional horror philosophy *Cyclonopedia*<sup>15</sup> are two notable examples of Wallace's successors. Both of these works draw on academia to induce Weird horror. In Danielewski's case, the spiraling authorship of the early internet provides a venue for the exploration of cognitive linguistics by way of the Weird mode. In Negarestani's, the fallout of the war on terror has laid bare material and existential horrors buried within the very soul of the Middle East. In both books, academic language and dizzying complexity are employed to systematically assault the narratives being constructed by states and systems in the early 2000s. Both authors utilize a maximalist, deconstructive style, linking them with Wallace. Together with many others, their writing represents a stylistically and modally cohesive reaction to the instability, uncertainty, and mutability of the two decades.

There are several other examples of upheaval periods and authors who have weirded their writing styles in response to the turbulence of their times, and the works discussed previously are just a few. When one studies the history of catastrophe, of cultural change, and of collapse, one finds artists turning to the Weird to express the strangeness of their times.

**ABSURDITY OF ALL KINDS OVERFLOWS FROM THE MOST BASIC OF HUMAN INTERACTIONS, SEEMING TO SPILL OUT OF THE SOCIAL AND INTO THE ACTUAL.**

## REFLECTION

With the basis for reactive Weirding established, what can we conclude about the future of American literature in response to more recent social, political, and medical changes? In some ways, the next Weird renaissance has already begun. *America and the Cult of the Cactus Boots: A Diagnostic* by Phillip Freedenberg revived the Wallacian maximalist format to comment on the bizarre psychosis of the Trump era. Ling Ma's *Severance* used a magical realist approach to elucidate the Chinese American experience of alienation and discrimination during the course of a pandemic two years before COVID-19. Science fiction authors like Ted Chiang have begun to Weird that subgenre, seemingly inspired by phenomena such as climate change and posthumanism.

The response to the COVID-19 pandemic is still incubating. It is too early to survey how extensive the reactive weirding of the American literary form might become. The creative artifact I produced in conjunction with this research, *The Weird Year: Things Made in Quarantine*, is a collection of reactively weird stories intended to capture my own experience of living through a pandemic. It also serves as a possible forecast of what may be to come for American literature if the process of reactive weirding takes hold of the mainstream.

In any case, literature will need to turn to new modes and new methods to make sense of what has

occurred. Old genre methods, developed in comparative stability, do not resonate as they once did. Transformation, mutation, and weirding are in some sense inevitable; what form American literature will take in the future remains to be seen.

## ACKNOWLEDGEMENTS

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### RAIN ETHERIDGE '22

Rain Etheridge '22 is an English major from San Antonio, Texas. In 2022, Rain won the Texas A&M Science Fiction and Fantasy Contest. Their experiences living around the world as a military brat have inspired an obsession with experimental and fringe literature. They plan to continue publishing their creative and critical work, while pursuing a teaching position.

# Lab-Grown Bone: Using Stem Cells to Successfully Replicate the Native Bone Healing Environment

*By Rithika Adavikolanu '22*

*Photo by Mehmet Turgut Kirkgoz on Unsplash.*

## INTRODUCTION

An estimated 2.2 million bone graft procedures are performed annually worldwide due to bone diseases, osteoporosis-related fractures, trauma, bone malformations, and tumor resections.<sup>1</sup> Autografts, where a patient's own bone is harvested from another site, are considered the golden standard for bone defect treatment; however, the limited supply and potential complications at the donor site remain a significant problem. Allografts, bone from cadavers, are a widely used alternative, but these pose a serious risk of disease transmission.<sup>1</sup> Bone tissue engineering seeks to develop better treatment alternatives for the large population with conditions that negatively impact bone healing.

The goal of bone tissue engineering is to fabricate scaffolds with the healing potential of autografts. A common strategy employs three-dimensional scaffolds, typically seeded with cells and other biological molecules, that promote desirable cellular interactions with their host to create an osteogenic environment. This technique is used to promote the formation of new bone to replace the scaffold.<sup>2</sup> Bone heals best when it is in an environment that closely resembles the native tissue. One way bone engineers replicate the native tissue environment is by coating scaffolds or implants with a cell-derived extracellular matrix (ECM), a complex mixture of proteins secreted by cells. The ECM resembles a bone that is developing or healing as opposed to an adult bone. For example, there are certain types of collagens (e.g., collagen VI and XII) found in developing bone that contribute to bone formation, but these are absent in adult bones.

Human mesenchymal stem cells (hMSCs) are one of the most common types of cells implanted on

these scaffolds. hMSCs have the ability to differentiate into multiple connective tissues (mainly bone, fat, and cartilage) and are found in high quantities within bone marrow, which is widely used for healing difficult bone defects. In addition, there is emerging evidence that the immuno-suppressive nature of hMSCs, along with their differentiation abilities, makes them highly attractive for regenerative techniques, as transplant rejections are a huge limiting factor for cell-based therapies.<sup>3</sup>

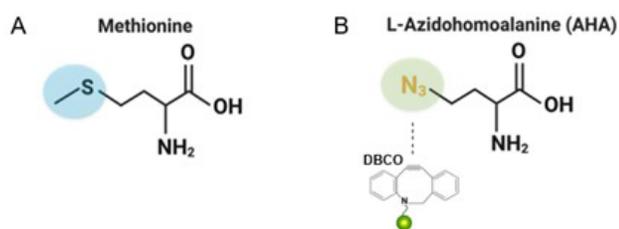
One way to coat scaffolds with ECM is to seed stem cells directly onto the scaffold, allow them to deposit a protein matrix, then remove the cells from the scaffold leaving behind just the matrix. However, this decellularization process can negatively affect the mechanical properties of the scaffold and the bioactivity of the ECM. This project investigates a method to covalently tether the ECM to scaffolds without compromising the integrity of either the ECM or the scaffold. We aim to achieve this through a process called click chemistry, which is a highly selective covalent reaction that occurs between two chemical groups (in our case, azide groups and alkyne groups) to “click” them together.

## METHODS

We used human mesenchymal stem cells (hMSCs), which have promising regeneration potential and are traditionally isolated from the bone marrow. However, hMSCs have limited proliferation abilities and high variation in potency depending on what donor or tissue they are sourced from. To bypass this, we derived our hMSCs from induced pluripotent stem cells (iPSCs) to produce induced pluripotent human mesenchymal stem cells (ihMSCs), allowing them to produce a plethora of cells originating from only one donor or cell source. We then introduced the small molecule GW9662 (GW) to our stem cells, which encouraged our cells to differentiate into bone cells. Importantly, the addition of GW also induces the secretion of ECM.

We utilized a process called metabolic labeling to incorporate an azide group, which is not naturally occurring in cells, into the ECM. We introduced our cells to L-azidohomoalanine (AHA), an amino acid

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**Figure 1. A)** A normal methionine amino acid with a typical sulfur group **B)** L-Azidohomoalanine containing an azide group (opposed to the typical sulfur group) shown binding to an alkyne group tagged with a fluorescent dye.

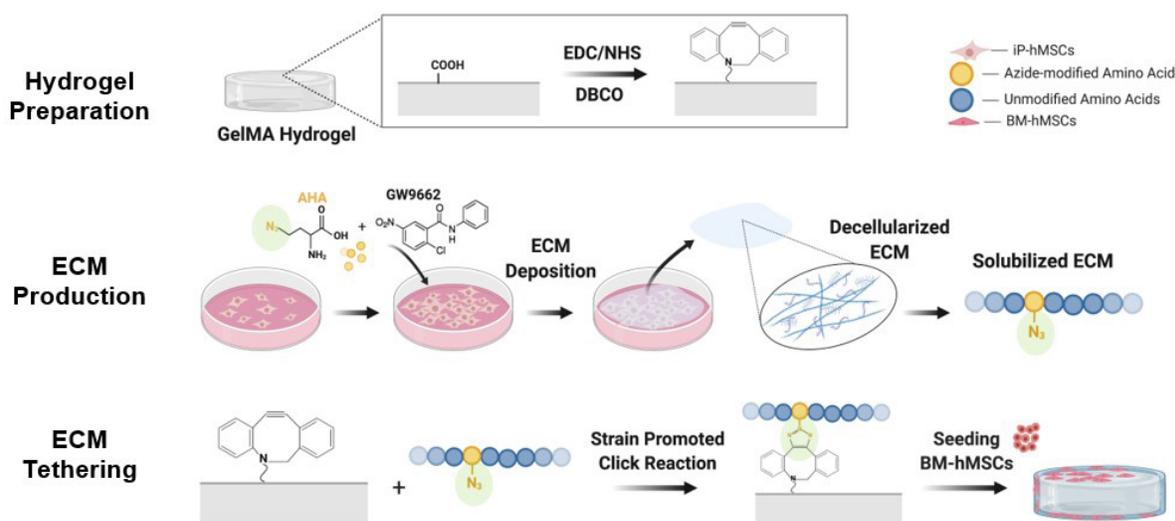
containing an important azide group (**Figure 1B**). Cells recognized AHA as an alternative to a methionine amino acid (**Figure 1A**) and thus incorporated it into protein synthesis, meaning that every new protein produced is labeled with AHA. Azides react very specifically with alkynes such as dibenzocyclooctyne (DBCO), thus we can track the deposition of proteins by adding fluorescently labelled DBCO. We are using this same chemistry to bind our azide-containing ECM to a scaffold labeled with DBCO.

We prepared our scaffolds using a hydrogel polymer, gelatin methacrylate (GelMA), creating it in a two-step process to produce a hydrogel scaffold labeled with DBCO (**Figure 2**, top row). ECM was

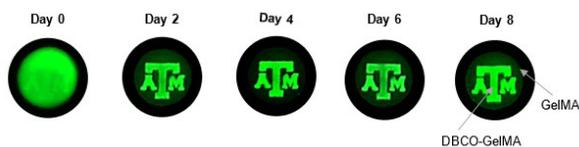
produced by introducing GW and AHA to our ihMSCs, which allowed the cells to differentiate into bone cells, encouraged the secretion of the ECM, and labeled our cell proteins with azide groups (**Figure 2**, middle row). The cells were allowed to grow for ten days to deposit an ECM made up of these newly synthesized, AHA-containing proteins. After the cells deposited the ECM, it was then extracted and the ihMSCs were removed. The decellularized matrix was consequently solubilized, leaving a protein stock to tether to the hydrogel scaffolds. Finally, we tethered the ECM to the DBCO-hydrogel scaffold by allowing the alkynes to react with the solubilized ECM containing AHA azides. The potency of the scaffold was tested by adding bone marrow derived hMSCs (BM-hMSCs) and measuring biomarkers for bone formation.

## RESULTS

Verification testing was performed to ensure the successful binding of DBCO alkynes to the hydrogel scaffold. Since alkynes have a high reaction rate to azide groups, we used a fluorescent dye linked to an azide group to visualize the presence of DBCO alkynes. We placed DBCO functionalized GelMA in TAMU molds with a plain GelMA outer circle. The



**Figure 2.** A visual depiction of the overall methods, with the first row illustrating the hydrogel preparation, second row illustrating the ECM production, and the third row illustrating the ECM tethering. Figure created using BioRender.



**Figure 3.** Plain GelMA and DBCO functionalized GelMA both placed inside a TAMU mold, treated with azide dye for two hours, and allowed to wash for up to eight days.

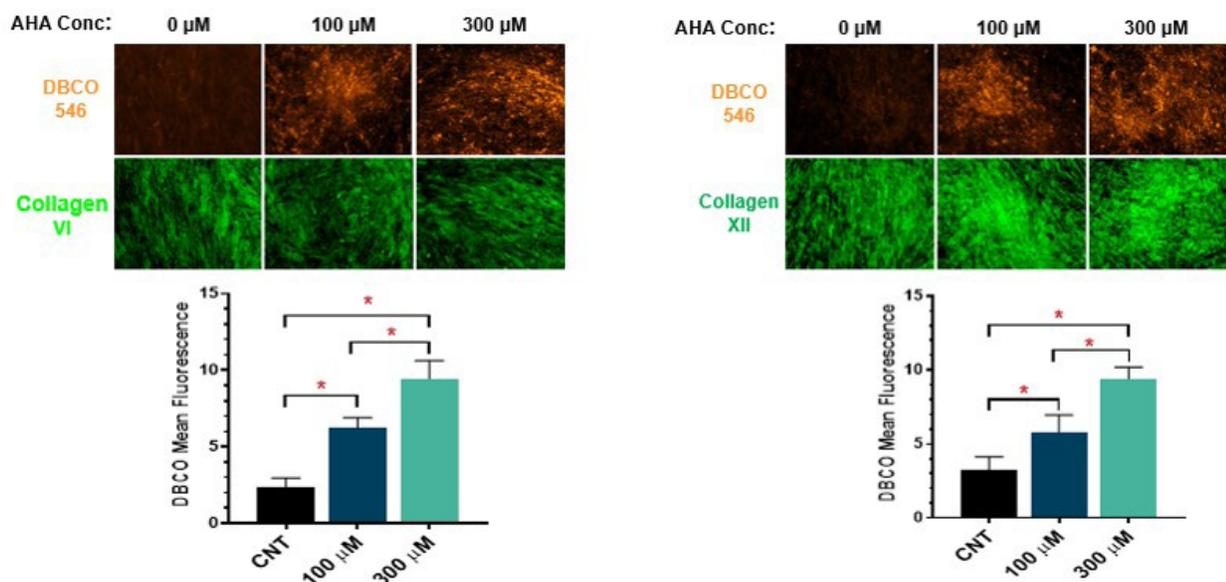
entire gel was dyed with an azide dye for two hours and allowed to wash for up to eight days to allow the unbound dye to completely wash away. After the conclusion of eight days, the DBCO functionalized GelMA was still fluorescent, indicating a stable reaction had occurred.

The ECM consists of high levels of collagen VI and XII, which play an important role in endowing osteogenic properties to the ECM. Therefore, when testing the properties of the ECM it is important to probe for the presence of collagen VI and collagen XII proteins to verify that the ECM is accurately replicating the healing bone environment. Furthermore, a major concern with click chemistry is that the composition and the function of the labeled proteins will be compromised. To ensure that feeding cells with AHA would

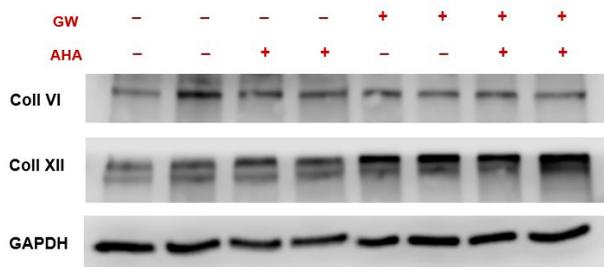
not alter the composition of the ECM, we analyzed the ECM monolayers grown in an increasing range of AHA concentrations from 0 to 300 micromolar. After ECM deposition, we performed fluorescence microscopy to stain for the presence of AHA. As shown in **Figure 4**, we found a direct relationship between AHA concentration and resulting fluorescence, proving that AHA was effectively incorporated into the ECM. Furthermore, the expression of both collagens VI and XII was unaffected by increased AHA concentration, indicating that AHA does not alter the composition of these important collagens.

Additionally, to determine the effect of AHA on the expression of collagen XI and XII, a western blot was performed (**Figure 5**); which is a common laboratory technique used to separate and probe for specific proteins in a sample of several different proteins. The same experimental groups described above were used for the western blot in duplicates. Results showed that the addition of AHA had no effect on the presence of collagens VI and XII within the ECM. This further demonstrated that the osteogenic properties of the ECM remained intact even after the metabolism of AHA.

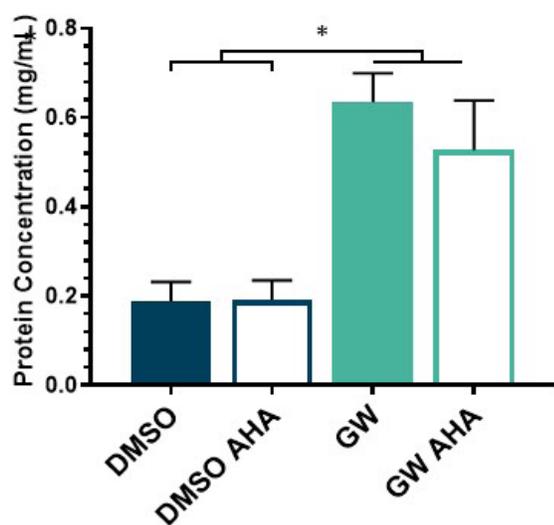
Finally, to determine if the GW and AHA added to the cells affected the protein yield in the ECM, we



**Figure 4.** Fluorescence microscopy done on ECM monolayer in increasing AHA concentrations from 0 to 300 micromolar for both collagens VI and XII proteins.



**Figure 5.** Western blot protein quantification bands for proteins of interest: Collagen VI, Collagen XII and GAPDH (housekeeping protein, loading control).



**Figure 6.** Quantification of protein concentration to observe the effect of GW and AHA on protein expression in the ECM. Four experimental groups were used: DMSO (control), DMSO and AHA, GW, and GW and AHA.

performed a bicinchoninic acid assay which quantifies the amount of protein present in each sample. We evaluated four groups: DMSO (control), DMSO and AHA, GW, GW and AHA for total protein expression present in each sample. Results showed that GW treated samples produced three-fold more ECM proteins than DMSO controls. This is a desirable outcome as GW was added to promote the secretion of the ECM and is therefore expected to yield a greater output of protein. Results also showed that the incorporation of AHA did not affect the ECM protein deposition, which is also desirable as the addition of a new metabolic group should not alter the inherent properties of the ECM proteins.

## CONCLUSION

Unlike current bone treatments, such as autografts and allografts, our method aims to replicate the natural process of healing bone rather than simply replacing damaged tissue with fully formed bone. Furthermore, by using actual ihMSC-derived ECM, we are able to preserve the richness of the natural bone healing environment and enhance the osteogenic properties of biomaterials. The goal of this research is to covalently tether the osteogenic ECM to a hydrogel scaffold without negatively affecting its osteoinductive properties. In this work, we have begun to apply metabolic labeling and click chemistry techniques to develop a novel biomaterial ECM-scaffold. A simple interpretation of these results leads to the conclusion that GW treatment provides an avenue to stimulate ihMSCs to deposit higher levels of ECM than DMSO controls. More importantly, our AHA (methionine substitute) can effectively be incorporated into the osteogenic ECM and we confirmed that AHA does not affect the number of proteins our ihMSCs produce or its composition (collagen VI and collagen XII). Our results show that ihMSCs seeded onto the decellularized, AHA-containing ECM experience the same osteoinductive effects observed from a standard osteogenic ECM. Our work thus far provides the foundation for an ECM labeled biomaterial decorated with DBCO, which we hope to achieve in the near future.

## ACKNOWLEDGEMENTS

I would like to thank Dr. Roland Kaunas for encouraging me to pursue various research opportunities and allowing me to take part in his research lab for the past four years. I would also like to thank my graduate mentors, Eli Mondragon and Berkley White, for their continued guidance and support throughout the course of this research project.

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## RITHIKA ADAVIKOLANU '22

Rithika Adavikolanu '22 is a biomedical engineering major from San Francisco, California. She has been involved in undergraduate research from 2018-2022, where she has studied various topics within the field of regenerative medicine. After graduation, Rithika will be researching host-virus interactions at UCSF before pursuing her Ph.D. in genetic engineering.

# Reaching

By Olivia G. Parker '22

## INTRODUCTION

My collage is a visual representation of perseverance and a celebration of what can be achieved by persisting regardless of difficulties. Collage is both a noun and verb that describes ways of gluing fragments of paper to various surfaces.<sup>1</sup> Collage was popularized in the early 1910s by modernist artists like Kurt Schwitters, Georges Braque, and Pablo Picasso. While there are different techniques that fall under the term collage, they are united by rejecting aestheticism and elevating everyday objects. Contemporary collage often explores humanity's relationship with time, nature, or the past through images or text.<sup>2</sup>

Similar in medium to the work of Kurt Schwitters, the collage is made from discarded paper material. His modernist, post-World War I collages used trash, broken objects, and other detritus arranged in careful harmony.<sup>3</sup> Some of his collages feature papers with no thematic connection to one another. Others, use the same types of paper. *Untitled (Quality Street)*<sup>4</sup> is made entirely from colorful candy wrappers and inspired my choice of materials. While Schwitters used found materials in his art to free them from the constraints of narrative and symbolism, my found materials are arranged into a specific symbolic image. Schwitters used abstraction to communicate that beauty and balance can be made from the rubble and debris of war. I turned away from abstraction and arranged my materials into a storytelling image.



**RIPPING UP MY  
HOMEWORK AND  
TURNING IT INTO  
AN ART PIECE WAS  
INCREDIBLY CATHARTIC.**

## METHODS

*Reaching* is a collage made of discarded homework. To source my materials, I asked my friends, co-workers, the people who lived in my dorm, and anyone I may have met in the last four years to donate a piece of homework from the most difficult class that they have finished at Texas A&M University. Handwriting in multiple colors of ink, highlighted sections, and feedback in red pen sprawls across hundreds of pieces of paper from lab notebooks, exams, notes, and final essays.

The inspiration for using homework in this piece came from a previous project completed in 2019, where I needed to finish a papier mâché mask by midnight and had run out of paper. I went through my bookshelf and found a previous semester's MATH 140 notebook. The spring of my freshman year was a difficult time and waking up for an 8 a.m. math class was especially challenging. I received a thirty-eight on the first exam, and a fifty on the second...but I could not drop the class because I needed to stay a full-time student. My friends tutored me, and I passed the class with a hard-fought grade of sixty-eight. Ripping up my homework and turning it into an art piece was incredibly cathartic.

While collecting papers from friends and acquaintances, I never asked for their backstories. Asking why a particular class was difficult felt too personal. However, everyone who donated homework shared their stories while handing me their papers. Some people just did not work well with a particular professor, or they found the course work overwhelming. One class was such a disparate match to a student's skills that they changed majors. Another student shared that the first assignment they turned in for a class was falsely flagged for plagiarism, and they had to fight the charge for the remainder of the semester. But everyone finished their class. They pushed through, did the work, and moved on to the next challenge. When I look at the jagged torn-paper mountain range in my artwork, I do not see a pile of struggles. I see a mosaic of triumph.

Initially, I was viewing these students as resilient. Academic resilience has been defined as “the

**BOTH RESILIENCE  
AND BUOYANCY ARE  
MAJOR FACTORS  
IN DETERMINING A  
COLLEGE STUDENT'S  
SUCCESS.**

heightened likelihood of success in school and other life accomplishments, despite environmental adversities brought about by early traits, conditions, and experiences.”<sup>5</sup> Students who find a class overwhelming for environmental or internal reasons and are successful despite them are demonstrating academic resilience. Academic buoyancy refers to everyday setbacks instead of challenging circumstances like low socioeconomic status.<sup>6</sup> Students who bounce back from failing a test, sleeping through a class, or surviving a plagiarism allegation demonstrate academic buoyancy. Both resilience and buoyancy are major factors in determining a college student's success. Most of the homework pages I collected are from students who demonstrated academic buoyancy.

## RESULTS

The source materials for this piece and their arrangement into a specific image can be read as a saying: Climbing the mountain is hard, but what you are reaching for is worth it. One of the most encouraging things that I heard in college was from a LAUNCH staff member. My thesis reviewer said, “What you are doing is hard.” Just the acknowledgment that the various tasks my fellow researchers and I were undertaking were difficult but still worth doing made all the difference and pushed me to keep climbing towards the answer to my research question.

Looking at my finished collage, I find comfort in knowing that MATH 140 was hard for me, but other people struggled in classes like PHYS 206, CHEM 120, or POSC 429. Each student who donated homework for the art piece passed their class, half of them have

graduated and several are going on to earn a higher degree. Sometimes it is encouraging to see that everyone has had ‘that class’ but are still successful. I hope that everyone who interacts with Reaching is inspired by these students’ academic resilience and buoyancy to push through whatever challenge they are facing.

## CONCLUSION

I see my artwork as a reflection of the goals of the *Explorations* journal and the concept of research. The figure reaching for the sky represents the curiosity of a single individual, who has pursued their academic interest through the highs and lows of a challenging journey. They have developed and demonstrated academic resilience and buoyancy to reach the highest point they can climb. Not satisfied by the plateau they have arrived at, the answer they found, or edge of knowledge, the researcher reaches out to discover more.

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### OLIVIA GRACE PARKER '22

Olivia Grace Parker '22 graduated with her Bachelor of Arts in performance studies and minors in English and film studies. She grew up as an Air Force kid, living in the Azores, Germany, Virginia, Alabama, Colorado, Pennsylvania, England, and Texas. Completing the LAUNCH Undergraduate Research Scholars program twice helped her define her artistic and academic research to focus on wonder, perseverance, performance phenomena, and transporting viewers to new worlds. This fall, she will start her Master's of Fine Arts program in acting and contemporary performance at Columbia College Chicago and Arthaus Berlin International School.

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Ellie is a sociology major from Arlington, Texas. She hopes to pursue a future career path that allows her to combine all her interests and skills in a way that sustains meaning in her everyday life.



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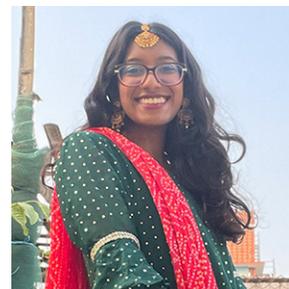
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