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About the Cover:

July 12th, 2023 marked the one-year anniversary of the James Webb Space Telescope's (JWST) first full-color images. As the largest and most powerful observatory, JWST was launched to uncover mysteries about the universe and its origins. The cover depicts the Carina Nebula and its "mountains" and "valleys" captured through JWST. Credits: NASA, ESA, CSA, and STScI.

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Testing the Effectiveness of the MotusApps LumiLave UV-C Device in Reduction of Microbial Load on Solid Non-Porous Surfaces.

Chae Hee Lim[1], Tatyana A. Sysoeva[1]

Faculty Advisor: Dr. Tatyana A. Sysoeva[1]
[1]*Department of Biological Sciences, The University of Alabama in Huntsville*

During the COVID-19 pandemic, the demand for disinfecting agents and devices to reduce microbial load drastically increased. Considering the diverse sources of microbial contamination of household items and food, a new device – LumiLave – has been designed to reduce microbial load on conventional store cashier belts. The LumiLave device uses the classic antimicrobial activity of ultraviolet illumination in the UV-C range to continuously decrease the number of viable microorganisms on moving conveyor belts. Here, we tested a prototype of this UV-C device to characterize its efficiency in reducing microbial loads on solid non-porous surfaces. To mimic contamination with bacterial pathogens, we used model Gram-negative and Gram-positive organisms, Escherichia coli and Staphylococcus aureus, respectively, as these species have a variety of pathogenic strains. A broad range of bacterial loads on glass slides were tested for viability upon application of the UV-C light with different exposure times. Resultant viability measurements, using colony counting, showed that the device robustly reduces bacterial load for both Gram-positive and Gram-negative species. 95-99% of bacterial cells were rendered non-viable in the first 30 seconds of exposure for both E. coli and S. aureus, within a broad range of initial densities of surface bacterial cells of 103-107 CFU cm-2. For more sensitive E. coli cells, 95% of killing was achieved within the first 15 seconds of exposure at the highest tested cell density of 108 CFU cm-2.

Introduction

The COVID-19 pandemic led to an increased demand for disinfecting agents and devices reducing microbial loads [6]. Following this outbreak caused by the viral pathogen SARS-CoV-2, the public has also become acutely aware of other microbial risks and bacterial contamination in everyday life. One potential source of microbial pathogens is routine grocery shopping, which can introduce new microbes from the store to food and other items brought into households. There is limited information on microbial load and composition of grocery store cashier belts. For comparison, microbial contamination of conveyor belts in different food-processing plants was characterized. Many microbes were found on conveyor belts from a salmon-processing plant, including the genera Listeria, Pseudomonas, Stenotrophomonas, Brochothrix, Serratia, Acinetobacter, Rhodococcus and Chryseobacterium, with a load up to 109 CFU cm-2 [5]. Even in environments outside food-processing plants, conveyor belts still experience contamination due to high numbers of microorganisms and foodborne pathogens, such as Listeria monocytogenes and Escherichia coli [1]. Poultry meat, one of the most handled produce in grocery stores, is also abundant in the Enterobacteriaceae and Staphylococcus species at approximately 4 and 17 CFU cm-2, respectively [4]. It is important to reduce microbes on conveyor belts at any given environment to prevent cross-contamination, a primary factor in the spread of foodborne illnesses [2].

Ultraviolet (UV) irradiation is known to have strong germicidal properties such as to sterilize or reduce the microbial load [8]. Lamps emitting 254 nm UV-C wavelength light [9] damage the deoxyribonucleic acid (DNA) of microbes when photons are absorbed. UV-induced formation of pyrimidine dimers between adjacent thymine bases disturbs the replication and accumulation of mutations. This process of UV germicidal irradiation may be used for air, water, and surface disinfection purposes [7]. In

fact, coronavirus aerosols, including those with SARS-CoV-2, have been found to have high susceptibility to UV-C light, which could be a prospective prevention mechanism for viral diseases [3, 10].

An abundant number of UV devices are available on the market due to their various applications, flexibility, and efficiency. The i-Robot UV-C, for example, has been introduced to disinfect rooms and equipment in hospitals, public transport, airlines, and enclosed spaces to reduce the spread of COVID-19 [3].

In this study, the new prototype UV-C device called LumiLave from MotusApps was tested for its efficacy. Standard quantification of viable test bacteria was used, – specifically, the Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus, as both species have pathogenic representatives and are implicated in the contamination of food chain products and surfaces of grocery store cashier belts.

Materials and Methods

Bacterial strains and growth conditions

Standard microbiological protocols were followed for bacterial growth and quantification for the selected test bacteria: Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus. In particular, the non-pathogenic E. coli BL21 (DE3) strain was grown overnight in 4 mL of Luria Broth (LB) or on LB agar at 37°C. The methicillin-sensitive strain of S. aureus ATCC 29213 was grown either for 2 hours or overnight under the same conditions and later plated in Tryptic Soy Agar (TSA).

LumiLave device testing

Experiments with the UV device were carried out in the Biosafety Cabinet (ThermoScientific 1300 Series A2) using microscope glass coverslips (Globe Scientific) placed in a 6-well plate (Fisher Scientific). Liquid cultures of the test strains in LB medium were applied to sterile glass cover slips, dried for a fixed amount of time, and exposed to the LumiLave prototype UV device. Each well was washed with 0.5 mL phosphate-buffered saline (PBS) solution after the exposure for quantification of remaining viable cells.

Quantification of viable bacteria

Serial dilutions were conducted for the cultures and PBS washes using a 96-well plate and 10 µL cells:90 µL LB dilutions. 2 µL of each dilution were plated in LB (E. coli) or TSA

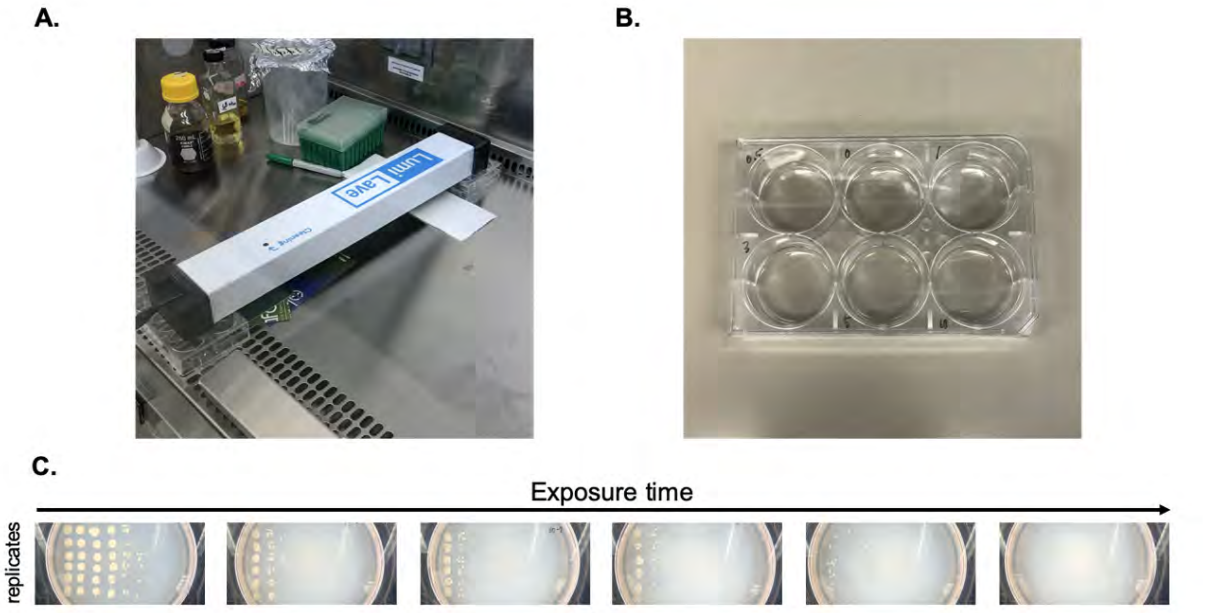

Figure 1. Testing and setting up the LumiLave device. A) Device prototype in the BSL2 Biosafety cabinet. B) 6-well plate holding sterile glass coverslips. C) An example illustrating viable cell quantification using colony counting protocol and rapid cell killing upon increased exposure times – compare the leftmost plate image to the adjacent one to notice a drastic reduction in cell viability in the first 0.5 min of UV exposure.

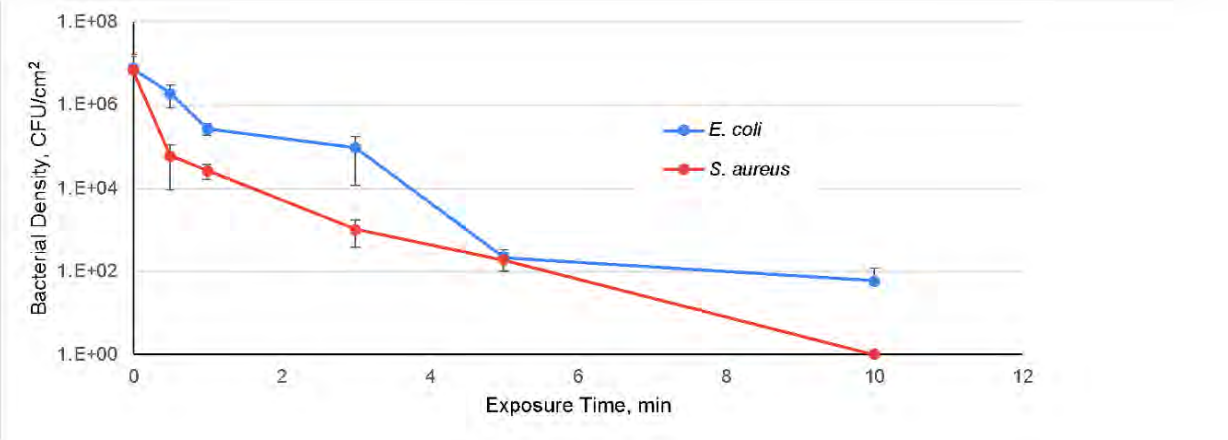

Figure 2. Initial E. coli and S. aureus viability under UV exposure. Cell densities are quantified as viable cells per mL of solution washing the surface. Initial cell load on coverslip was ~10^8 CFU to 22 mm x 22 mm surface. Error bars represent standard deviation for three technical replicates for each experiment. Note that the data are presented in the log10 scale on the Y-axis. 23 mm from light source to bacteria on glass surface.

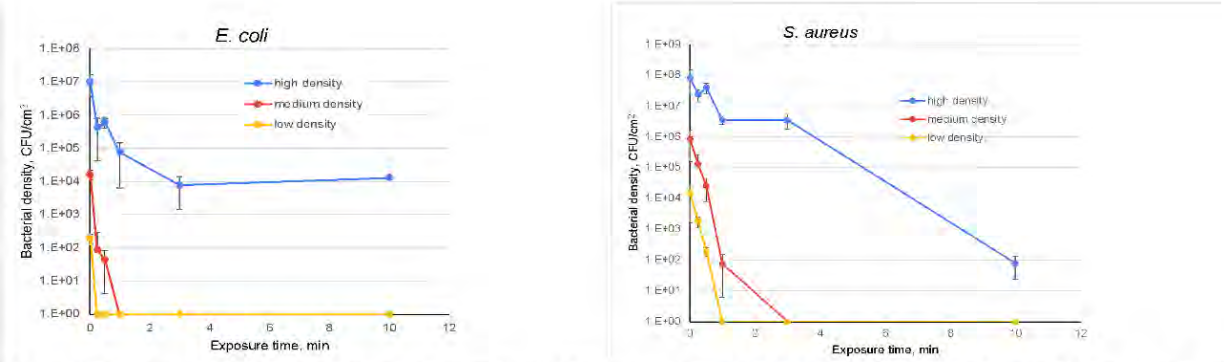

Figure 3. *E. coli* and *S. aureus* viability under LumiLave UV exposure. Bacterial densities are quantified as viable cells per mL of solution washing the surface. Initial bacterial load on coverslip was ~108, 105, 103 CFU per 22 mm x 22 mm surface and indicated on plots as high, medium and low densities. Error bars represent standard deviation for three technical replicates for each experiment. Note that the data are presented in the log10 scale on Y-axis. These sets of experiments were done at a 25 mm distance from the UV light source to bacteria on the glass surface.

(S. aureus) agar using a multichannel pipette. After overnight incubation at 37°C, colony forming units (CFUs) for each plate were counted and calculated to result in a CFU/mL value by multiplying by the dilution factor. The average CFU/mL was obtained from the 6 repeats for each time point. Standard deviation was calculated to estimate the noise in the CFU/mL measurements.

Results and Discussion

Developing experimental setup for viability measurements

The possibility of quantifying viable bacteria on glass coverslips and their survival upon UV exposure was first tested. A "no treatment" control was used along with a 20-minute exposure to the Biosafety Cabinet (ThermoScientific 1300 Series A2) UV light that was previously tested to sterilize exposed surfaces. This experiment was done for belt material squares (~1 sq inch) and glass coverslips. The results showed that: (1) Belt material is hydrophobic and does not allow for easy application of bacteria without residual liquid; (2) Bacterial cells on the glass surface have reduced viability upon prolonged (over 30 min) drying times.

As cashier conveyor belts are not usually covered in a thick layer of aqueous solution, which ultimately serves as a protectant for the bacteria by absorbing UV, glass coverslips were used for all further experiments. Based on this experiment, the drying time for bacterial cells loaded on glass coverslips was

fixed to 10 minutes throughout the initial experimental setup as described below.

Testing initial exposure times

10 µL of cell suspension was applied to glass coverslips placed in a sterile 6-well plate, which was then dried for 10 min and exposed to 0, 0.5, 1, 3, 5, and 10 minutes of LumiLave device's light. The device was placed on support to set a distance between the inoculated surface and the lowest edge of the device as 23 mm. After the exposure, each coverslip was flooded with sterile PBS solution (0.5 mL) to elute viable cells. Obtained cell suspension was serially diluted to 107 using ten-fold dilutions and plated for colony forming units (CFU) counting by applying 2 µL to LB or TSA agar plates in 6 replicates. These plates were incubated overnight at 37°C and formed colonies for counting after the 24-hour incubation. These initial experiments showed that the main reduction in surviving bacterial cells occur within the first minute of UV exposure, and therefore, the next sets of experiments were done with an additional 15 second (0.25 min) time point (Fig. 2).

E. coli and S. aureus viability under LumiLave exposure

Using conditions identified above, we conducted experiments with dense overnight cultures of both test strains that was equivalent to loading ~108 CFU to a 22 mm x 22 mm surface. Exposure times of 0, 0.25, 0.5, 1, 3, and 10 minutes at a 25 mm distance were used. The results show that up to 99% of all cells are killed in the first half of a minute of UV exposure (Fig. 3, blue).

E. coli and S. aureus viability at different initial cell densities

Next, different initial cell densities were tested on coverslips with more realistic medium and low densities of E. coli and S. aureus cells – around 105 and 103 CFU per 22 mm x 22 mm surface (Fig. 3, red and yellow). From these results, it is also notable that the killing efficiency strongly depends on initial surface contamination with bacteria. These experiments were repeated twice with triplicates on each day.

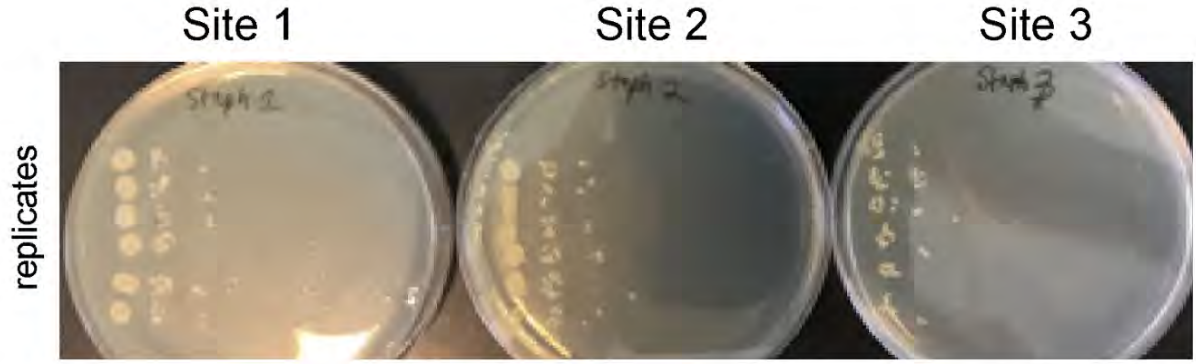

Figure 4. Prototype LumiLave device shows unequal UV output as judged by killing.

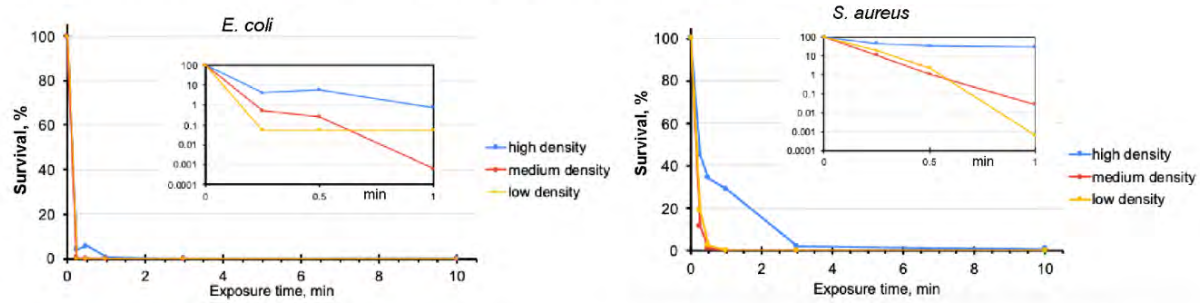

Figure 5. Survivability plots for *E. coli* and *S. aureus*. Inserts show the first minute effect of exposures at different initial densities.

Testing uniformity of exposure under the UV lamp

In a couple of our preliminary measurements, a high level of noise in colonies' numbers were noted. Therefore, we tested several locational positions under the LumiLave lamp to see if illumination unevenness might explain the observed variability. Two preliminary runs showed that one out of the three selected spaced positions showed a different effect in killing from the first two positions (Fig. 4) suggesting the lack of UV illumination uniformity. Based on this result, further measurements were done at a fixed location.

Limitations of the current study

At this point, the average bacterial densities at the cashier belts are not known, but it is safe to assume that they are lower than the microbe-rich belt surfaces in the food processing plants. But even in those settings, many estimates put usual bacterial loads between 101-105.6 CFU cm-2 (Table 1 in [5]) and only established biofilms show higher bacterial densities. This suggests that our experiments use bacterial densities several orders of magnitude higher than those observed outside of the laboratory setting. Therefore, we conclude that the tested device provides a significant reduction of viable bacteria on solid surfaces suitable for practical applications.

This study only addresses the viability of two bacterial species and did not use pathogenic strains of these species, which might be considered a flaw. It also only addresses bacteria and no other types of microbes such as viruses, fungi, and archaea. Nevertheless, the UV antimicrobial properties are well established and presented here, and this finding might serve as a rough approximation of the general antimicrobial properties of the new device. Specific measurements on other types of microbes need to be done to establish quantitative properties.

We lack proper approximation of the range of conveyor belt rates and therefore, it is not possible to estimate how many passes will be required to achieve a certain degree of microbial load reduction. We also used glass slides as an approximation of the conveyor belt surface. The results obtained are applicable only to completely dry surfaces as the presence of a water layer will drastically reduce the amount of UV light reaching the surface, protecting the microbial cells.

Conclusions

Based on the results presented here, UV survival can be calculated as a ratio between cell counts for the irradiated (certain exposure time) and unexposed control samples (0 min). The results show that within the first 15 seconds, 96 to 99.9 % of E. coli cells are killed while 55-81% of S. aureus are eliminated in the same time frame (Fig. 5). This discrepancy is expected due to the more intrinsically protected Gram-positive bacteria such as the S. aureus. Nonetheless, at 30 seconds, 95-99% of cells are rendered non-viable for both E. coli and S. aureus bacteria, within a broad range of initial densities of surface bacterial cells from 103 to 107 CFU cm-2, suggesting that the LumiLave UV-C device is efficient for the intended application of continuous microbial load decrease.

Conflict of interest statement

This study was sponsored by MotusApps.

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Effects of Loneliness on Brain Structure Across the Lifespan

Kyiler Bolton[1], Hannah L. Apostolou[1], Dr. Ian McDonough[1]

Faculty Advisor: Dr. Ian McDonough[1]
[1]*Department of Psychology, The University of Alabama, Tuscaloosa, AL 35487*

Loneliness is a neurological epidemic impacting adults throughout their lifespans. Regardless of age, those who are socially isolated have a statistically higher chance of cortical thinning, cognitive impairment, and decaying brain structure. Recent studies have shown similar correlations between higher levels of loneliness and damaged regions of the brain. However, we found inverse results in our study. Where we tested loneliness and age as an interaction against three regions of interest. Statistical variations from 87 participants in this study will not only show significant interactions between age and loneliness with brain structure growth in the pericalcarine gyrus for older adults, but brain structure decay in both the right superior temporal gyrus and the left superior frontal gyrus for younger adults.

Introduction

The study of loneliness and its relation to cognitive decline has been a rapidly expanding area of interest. Humans depend on social interaction to stimulate their brains and keep intelligence acute. Hence, loneliness can have various consequences on an individual's brain. For example, chronic loneliness can lead to impairments in attention, cognition, affect, and overall behavior. The loss of cognitive function can even remove individual's ability to live independently. Along with the individual implications, chronic loneliness has been seen to affect morbidity and mortality. Compared to other age groups, older adults may be particularly susceptible to the effects of loneliness because they transition from a period of social comfortability to social unfamiliarity [9].

Loneliness in older adults speeds up the aging process and negatively impacts cognitive function [9]. Cognitive impairment is thought of as an individual's general memory loss. A gradual decline in one's aging mind and overall neurological performance. Where we start to see the early onset of diseases like dementia and Alzheimer's. Mild cognitive impairment (MCI) can quickly lead to dementia or Alzheimer's disease. Researchers have no definitive cause of MCI in the brain. However, the risk of contracting MCI rapidly increases as the mind ages. MCI is defined as memory loss or other cognitive ability loss in individuals who still can maintain most of their independence in daily activities. It is thought that up to 20% of those with MCI are likely to develop dementia or Alzheimer's [18].

Loneliness is a universal emotion. Emotions in the brain are held in the amygdala and important to how the brain interacts with itself in different regions. Loneliness is a negative emotional that can easily influence brain atrophy and cognitive decline. Negative impacts on brain structure like cognitive impairment and age can influence diseases like dementia and Alzheimer's [5].

Cognitive Impairment and Brain Structure

Cognitive impairment may indicate that an aging mind is declining. Loneliness has been reported to play a large role in the manifestation of cognitive decline. For example, Cheng and associates (2018) found that neuropsychological testing performance in an MCI group of older adults was significantly worse than that of a cognitively normal group. Furthermore, significant thinning was found in the anterior cingulate and superior temporal regions of the MCI group compared to the control. The study was able to show significant results in the atrophy rate of the occipital region in subjects with Alzheimer's disease and MCI [4]. The

occipital region of the brain is one of the four lobes of the brain where the peri calcarine gyrus is housed.

Brain Structure

Moreover, it is important to consider how impairment to the superior temporal gyrus, superior frontal gyrus, and peri calcarine gyrus influences cognition. A study conducted by Ye Zhang, and his associates (2022) studied the influence of perceived social isolation or loneliness on brain structure and future cognitive outlooks for those who currently had or were at risk for Alzheimer's. The experiment included 176 elderly patients (Mean age: 78), 39 subjective cognitive decline, 53 mild cognitive impairment, and 84 with Alzheimer's. It was found that those patients with MCI showed decreased regional gray matter volume in the left middle occipital gyrus, which suggests that loneliness can alter brain activity in visual, attentional, and emotional processes [23].

Superior Temporal Gyrus (STG)

The STG is located at the topmost part of the temporal lobe. The STG functions with both hemispheres of the brain. The right hemisphere acts as a site of convergence and processes both object and spatial information. Recent fMRI studies have also recorded the importance of social perception in the STG. In the left hemisphere, the STG mediates language processing, perception, the production of speech, and is involved in auditory short-term memory [12]. The STG also houses the amygdala. The amygdala is a major processing center for emotions, and most important for our study, seems to link emotions to memories [21].

Superior Frontal Gyrus (SFG)

On the other hand, the SFG is the medial-most gyrus on the frontal lobe and makes up one-third of the frontal lobe. The SFG controls higher cognitive functions and is known to play a significant role in working memory [7].

Pericalcarine Gyrus

The pericalcarine gyrus is a very small region of the brain located on the medial surface of the occipital lobe and divides the visual cortex. Functionally, the calcarine cortex or striate cortex is essential for basic attributes of visual scenes, determining orientation, spatial frequencies, and color properties of visual stimuli [6].

Similarly, studies have reported correlations between cognitive impairment and cortical thickness. Cortical thickening is related to MCI and poor performance on neuropsychological performance tests [4]. Cortical thickness is the measure of the width of an individual's gray matter in the cerebral cortex. Cortical thinning is the process by which gray matter width in the cerebral cortex begins to diminish. Decreases in cortical thickness are related to both age and MCI. The diminishing of this gray matter is an indication of impaired intelligence and compromised cognitive function.

Difference of Loneliness in Younger versus Older Adults

Loneliness presents itself differently throughout the lifespan. However, we speculate that younger adults facing transient or chronic loneliness may show even greater signs of brain structure decay than older adults. Hawkley and partners (2022) identified similar risk factors in younger adults that are also seen in older adults. Non-lonely younger adults are shown to have more contact with friends than older adults, so contact was strongly related to loneliness for younger adults. Another factor affecting younger adults is work status, where work status is strongly related to loneliness. Though, intuitively, younger adults struggling with close relationships are going to experience differing stages of loneliness, data from recent studies support the claim that younger adults are lonelier than older adults. These were among the first researchers to show how loneliness is distributed across the age range of adult populations [8]. We can use the data from this study to find authenticity in our observation of loneliness and age as an interaction.

Present Study

The goal of the present study is to examine the effects of loneliness on brain structure across the lifespan. We predicted that loneliness would be negatively related to brain structure in aging participants. This study was

designed to be exploratory in nature using a secondary data analysis method to understand how brain structure can be correlated with loneliness and age. With the confidence that our literature may one day nurture a desire for more studies regarding brain structure and emotions across the lifespan.

Methods

Participants

This study included 87 participants in the age range of 20 years old to 74 years old drawn from the Alabama Brain study on Risk for Dementia. Several factors were considered when excluding certain populations from the participant pool. Potential candidates were excluded if diagnosed with dementia or if they scored 20 or less on the Saint Louis University Mental Status Exam (SLUMS), as these scores indicate a potential for dementia [16]. Lastly, those with a history of cerebrovascular accident (CVA, commonly referred to as a "stroke"), those who have experienced traumatic brain injury, and those with a history of substance abuse were also disqualified from the study. The final participant sample included 87 adults with ages varying between 20 years old and 74 years old (M = 51.75, SD = 17.23).

Measures

To gather data for this study, A whole brain analysis was conducted. Participants then underwent a battery of neuropsychological tests. Along with this, participants answered a series of questions regarding their health and emotional, psychological, and socio-cultural information. Notably, the UCLA-R was used as one of the most reliable data sets in this study. The UCLA-R is a 20-item self-reported assessment measuring feelings of loneliness and social isolation. It is known as subjective in its measure, but it will allow participants to report their exact level of loneliness at the time of the experiment. Researchers have concluded that the measure is highly reliable in both internal consistency and test-retest reliability. Construct validity was also confirmed by relations with measures of individual's interpersonal relationships and correlations between loneliness and measures of health or well-being [15].

Procedure

Using FreeSurfer version 6.0, certain structural brain pictures were preprocessed. The Freesurfer recon-all pipeline applied several transformations to the raw imaging data, such as bias correction, skull stripping, affine registration onto the Talairach atlas, and removal of the brainstem and cerebellum. During the creation of the gray matter boundary and pial reconstruction process, mind control was utilized to identify outlying brain volume or cortical thickness values. To remedy any inaccuracies, manual corrections were implemented. Mean gray matter volume and cortical thickness values for each network were extracted using surface-based network templates from. All estimates of gray matter volume were regressed to remove intracranial volume before being used in our primary analyses [1]. Microsoft Excel was used to materialize data for basic statistical measures and create figures from significant results for visual reference. Where the measured value of loneliness for a specific participant at a specific age was correlated with the specific measured values of the STG and SFG regions for that participant.

Results

The figures provided below show values of statistical importance. For all ages, greater loneliness was associated with larger volume and surface area in the lateral occipital and peri calcarine gyrus at cluster correlated p-values < 05. There was no significant interaction found between our regions of interest and the measure of thickness. Figure 1 shown below highlights the pericalcarine gyrus area of the brain.

Figure 1. A whole-brain map of the positive relationship between loneliness and brain volume/surface area in the peri calcarine gyrus. Values are significant at a cluster-corrected p-values < .05.

The interaction of age and loneliness concerning the volume and surface area of the peri calcarine gyrus led us to believe that the

brain structure of older adults is increasing. The figure above depicts a brain map of where statistical calculations showed regions of increased brain structure and possibly even increased brain development in the highlighted areas. The superior temporal gyrus and the superior frontal gyrus also showed interactions with age and loneliness in volume surface area. A statistical interaction is the reliance of one variable on multiple others. Below are figures representing the interactions that occurred in samples of middle-aged to older adults and younger adults.

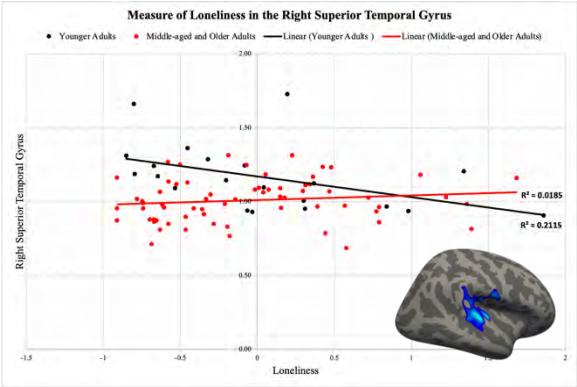

Figure 2. Loneliness is represented on the x-axis and the right superior temporal gyrus brain region is represented on the y-axis. Twenty-one participants in the young subgroup (aged 20-30) are represented by the black clustered data and sixty-six participants in the middle-aged to older subgroup (aged 50-74) are represented by the red clustered data. Two trendlines have been added to give a linear representation of the best-fitting data points. The R^2 values associated with the trendlines intend to prove reliability.

Figure 2 shown above reported two slightly different coefficients of determination. The 66-participant middle-aged to older adult sample with interaction in the right STG region of the brain returned a value of 0.018 or 1.8% variation. The 21-participant young adult sample interaction returned a value of .211 or 21.1% variation.

Similarly, figure 3 represented the measure of loneliness in the left SFG. The 66-participant middle-aged to older adults' sample reported a value of .0517 or 5.17% variation. Whereas the 21-participant younger adults sample reported a value of .082 or 8.2% variation. Interpretation of this graph shows similar results to Figure 2. Where middle-aged to older adults showed a slight trend of positive correlation. However, younger adults in the left SFG only showed a slight negative correlation in brain structure related to loneliness compared to previous results in the right STG that were noticeably more significant. Meaning that younger adults experiencing greater loneliness are showing smaller surface area and volume in the left SFG.

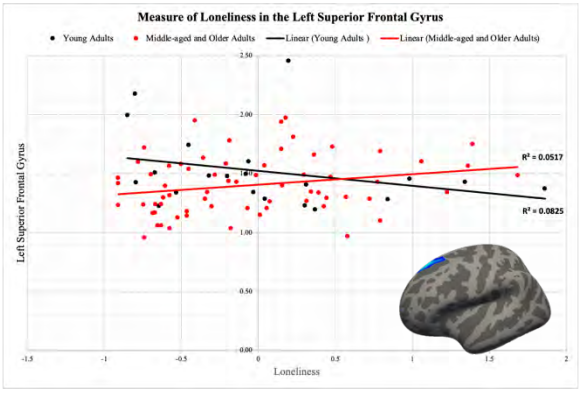

Figure 3. Loneliness is represented on the x-axis and the left superior frontal gyrus brain region is represented on the y-axis. Twenty-one participants in the young subgroup (aged 20-30) are represented by the black clustered data and sixty-six participants in the middle-aged to older subgroup (aged 50-74) are represented by the red clustered data. Two trendlines have been added to give a linear representation of the best-fitting data points. The R^2 value associated with the trendlines intend to prove reliability.

Discussion

We predicted that loneliness would be negatively related to brain structure in aging participants. Findings at the in our data suggest that we have reason to believe that there is significance to be found in the interaction of loneliness and age for all three of our ROIs ($p < 0.05$). This explains why we can use the literature mentioned above to support evidence of increases in brain structure in those older participants with loneliness. Particularly in figure 1 depicting the peri calcarine gyrus where the measure of loneliness and age interaction may mean that loneliness is stimulating the brain. Growth in the occipital cortex could be a sign of better visual recognition with faces or objects and memory formation. Meaning that older individuals could be stimulating this region of the brain by watching, reading, or seeing certain activities.

Zhang's findings from the experiment noted above greatly support our findings in the pericalcarine, STG, and SFG areas of the brain. Where they found that brain activity in visual

(peri calcarine), attentional (SFG), and social perception (STG) was affected by loneliness. Findings from this study can lead us to assume that the participants from our study may show a steady decline in brain structure with increased loneliness.

Interpretation of this data assumes that there is a significant negative correlation with loneliness and age in the right STG for younger adults reporting high levels of loneliness. Younger adults experiencing greater loneliness are showing smaller surface area and volume in the right STG. There was little to no statistical variation for the middle-aged to older adult group. The results showed a very small positive correlation but was not statistically significant for measures of brain structure.

The decline in brain structure of the younger adult population in our study could have various explanations. Younger adults in our study show the greatest association between loneliness and brain structure. Brain map regions highlighted in blue on figures 2 and 3 show two brain regions heavily involved in cognition. Particularly right STG region which can be associated with the amygdala. The amygdala is important in helping us understand how the younger adult mind processes emotions. It is our belief that younger adults lack the emotional regulation skills that older adults have developed throughout the lifespan [16].

Findings in the left SFG region can be associated with higher brain function. This region of the brain lies in the front lobe and is thought to contribute to both higher cognitive functions and the working memory. However, there is still an ongoing debate as to what exactly may be happening in this area of the brain. Working memory is important for us because it allows us to retain a small amount information about our observable environment and stored memories and use it during the execution of other tasks, we are engaged in [20].

This evidence supports the claim made earlier that loneliness could be increasing brain structure in older adults. Inversely, there is a negative association with the measure of loneliness in the right STG and left SFG for younger adults. Meaning that young adults in this experiment recorded significant results for changes in brain structure. From these findings, we can then assume that the effects of loneliness increase brain structure in older adults and decrease brain structure in younger adults. However, these finding seem to be contradictory of our original thoughts. Where most published scientific studies have found that more negative correlations with loneliness and age are often represented in older adults.

The lack of statistical significance in a portion of our data is problematic. All the measures calculated only returned cluster-correlated p values of < .05. This indicates a much weaker statistical correlation between variables than at the p <.01 range. A larger sample size and a broader range of ages in the young adult cohort would have produced more reliable data. Along with sampling issues, the UCLA-R neurological assessment is also slightly bias. Participants can be subjective on the UCLA-R questionnaire. They may be experiencing different levels of loneliness at the time of the test and could record different results on separate days. A possible solution to this issue could be double testing on separate dates or a less subjective measure of their loneliness.

Conclusion

This study is a great example for future studies in loneliness because the correlations we found between the loneliness and age interaction on brain structure deterioration in younger adults could be further examined. Though a large portion of the discussion section was speculation, we believe that a great deal of the core information provided by this study could be pertinent to the mystery of the young adult mind. We hope our findings will contribute to the rapid advancement of neuroscience and influence others to study how brain structure is impacted by different factors during the lifespan.

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Exploring the Interaction Between Black Hole Feedback and Cold Fronts in Galaxy Clusters

Isabelle Wingate[1], Dr. Stephen Walker[1]

Faculty Advisor: Dr. Stephen Walker[1]
[1]*Department of Physics and Astronomy, The University of Alabama in Huntsville, 301 Sparkman Drive, Huntsville, AL 35899, USA*

X-ray observations of nearby galaxy clusters have shown evidence of black hole feedback through X-ray cavities, or bubbles, blown up by such feedback. Some galaxy clusters have also gone through a minor merger event that causes a sloshing of the gas at the core center, known as a cold front. We studied nine galaxy clusters, observed with the Chandra X-ray telescope, which feature both cold fronts and X-ray cavities, to explore what fraction of them have bubbles that survived going through a cold front. We observed with Chandra nine galaxy clusters featuring both cold fronts and bubbles to determine the fraction of such clusters whose bubbles survived going through a cold front. We found that eight of the nine galaxy clusters did not have bubbles outside of the cold fronts. However, one of the nine, the Ophiuchus cluster, has what could be a bubble outside of its cold front. If it is a bubble, it has very unique properties, as bubbles typically come in pairs located opposite each other. This potential bubble also has an extremely large size, which makes it unusually powerful. We used a new filtering technique known as the Gaussian gradient magnitude (GGM) to emphasize small details that, when applied to the Ophiuchus cluster, did not indicate a bubble [7]. Due to these findings, it is more probable that this observation is a feature of the intracluster medium rather than a bubble.

Introduction

At the center of galaxy clusters there is a supermassive black hole. When material, such as gas, falls into the gravitational field of this black hole, it forms an accretion disk around it. This can produce jets of strong radiation and outflows of material. These outflows have a significant impact on the surrounding gas and stars in the galaxy. These outflows can heat or cool the gas of the surrounding galaxies, promoting or suppressing star formation in a process known as feedback. The hot intracluster medium (ICM) between galaxies displays temperatures of hundreds of millions of Kelvin, meaning that it emits in X-rays. From an observational point of view, the clearest indication of the effects of black hole feedback on the cluster scale is the existence of large X-ray cavities, or bubbles, maintained by the energy input of the central black hole [3]. This process can be observed by the *Chandra* X-ray Observatory.

Isolated galaxies do not show the effects of this feedback because they lack a medium around them for the jets to push against. When two galaxies sideswipe each other, it produces a spiral-shaped structure known as a cold front around the central galaxy. A cold front is a sharp boundary between two regions of gas with different temperatures. The shear between the gas can cause wave-like features, known as Kelvin-Helmholtz (K-H) instabilities, which can grow over time. The presence of a cold front in a cool core could indicate a movement of gas and a possible deviation from hydrostatic equilibrium. It is important to understand the characteristics of such a wide-ranging phenomenon, as it is necessary to characterize the dynamics of these galaxy clusters. Bubbles should rise out through the cold front, but at present it is unknown what actually happens when a bubble interacts with a cold front [8]. Simulations of rising bubbles in cold fronts of cool-core clusters suggest that bubbles may be destroyed by the cold fronts, but this process has yet to be observed [4].

Methods

2.1 X-Ray Data

Chandra has accumulated a vast archive over the 21 years it has been in operation. For this project, we wanted high quality data to construct a sample of galaxy clusters. To do this, we selected bright nearby clusters ($z < 0.1$) that cover the full mass range of galaxy clusters. We cross-referenced a paper with a list of X-ray cavities in galaxy clusters with another paper on cold fronts in galaxy clusters to create a group of galaxy clusters that meet both conditions [2], [5]. This resulted in the selection of nine galaxy clusters for study: Centaurus, Perseus, Ophiuchus, A2199, A262, A1795, 2A0335, A2029, and A85. Ophiuchus was specifically chosen due to the recent discovery of a giant radio fossil potentially caused by the most powerful feedback recorded thus far [6].

We downloaded approximately 150 kiloseconds of observation time per galaxy cluster from the *Chandra* Data Archive. The observations were made with the Advanced Charged Coupling Device Imaging Spectrometer (ACIS) due to its high-resolution imaging. *Chandra* has a spatial resolution of half an arcsecond, which means that observations not aligned on the center of the ACIS chips will blur and spread out the photons. These observations can result in worse quality stacked images, so it is better not to use them to get a clearer image. We discarded any of these low-quality observations to ensure that the images would have the best resolution possible.

We used the *Chandra* Interactive Analysis of Observations software (CIAO 4.13) and the High Energy Astrophysics Software (HEASoft) to clean up the observations. Specifically, we reprocessed the images using CHANDRA_REPRO, stacked the images with REPROJECT_OBS, and then extracted the images with FLUX_OBS. As the bubbles rise out of the galaxy cluster, they become progressively more difficult to see. We filtered the images using a new technique called the Gaussian Gradient Magnitude (GGM) filter [7]. The GGM filter enhances small features by measuring the gradient in surface brightness, which makes it possible to see faint bubble rims that would usually go undetected in the original image. We used FIMGMERGE to create a single stacked image from multiple observations with varying σ values of the GGM filter. The σ parameter is the Poisson error in the mean count rate. GGM images with lower σ values will detect the finest structures in the center, whereas higher σ values help to pull out the large-scale features in the outskirts. This allowed us to easily find the cavities and measure how far out they were from the center core.

The time scale in which these bubbles move out is hundreds of millions of years. We can estimate the age of the cavities because they travel at the speed of sound, which can be calculated by the sound crossing time:

$$t = \frac{R}{c_s} \tag{1}$$

where

$$c_s = \sqrt{\frac{kT\gamma_{ICM}}{\mu m_p}} \tag{2}$$

and $\gamma_{ICM} = 5/3$ is the adiabatic index of the intracluster medium, $\mu = 0.62$ is the mean atomic weight, m_p is the proton mass, and kT is the temperature of the galaxy cluster. We used the Archive of Chandra Cluster Energy Profile Tables to obtain the temperature of each galaxy cluster [1]. The results are shown in Table A1.

2.2 Sloshing Simulations

We used the Galaxy Cluster Merger Catalog to download the simulation data of the sloshing of magnetized cool gas in the cores of galaxy clusters [10]. The magnetization of the galaxy clusters plays a vital role in the development of the cold fronts, as well as K-H instabilities. The plasma of the magnetic field is the ratio of the thermal and magnetic pressures:

$$\beta = \frac{p_{th}}{p_B} \tag{3}$$

where p_{th} is the thermal pressure, $p_B = B2/8\pi$ is the magnetic pressure, and B is the magnetic field strength. β is a high value that usually ranges from 100 to 1000, with lower values indicating a stronger magnetic field. If the magnetic field strength of the cluster is high enough, the ability of K-H instabilities to grow is suppressed. It is also true that K-H instabilities are more likely to form at high radii because the magnetic field is weaker. Figure 1 shows the cold front that forms up to 3.20 Gyr with β =

200. We used this information to find the relationship between the size of the cold front and its age, as shown in Figure 2. This relationship made it possible to calculate the age of each cold front, which is shown in Table A1.

Results

Figures. 3, 4, and 5 show the stacked and reprocessed images and the GGM images of each galaxy cluster. Figure 3 shows four galaxy clusters with bubble pairs opposite each other. Figure 4 shows that four of the galaxy clusters only have bubbles appearing on one side, as opposed to bubbles 180° from each other. All of the galaxy clusters besides Perseus have only one pair of bubbles. In the Centaurus cluster, the left bubble is clearly distorted and appears to have traveled through a slightly more developed part of the cold front. Looking closer at cluster 2A0335, there are two adjacent bubbles, one being slightly further out. There should be four bubbles total, but it would seem that the missing

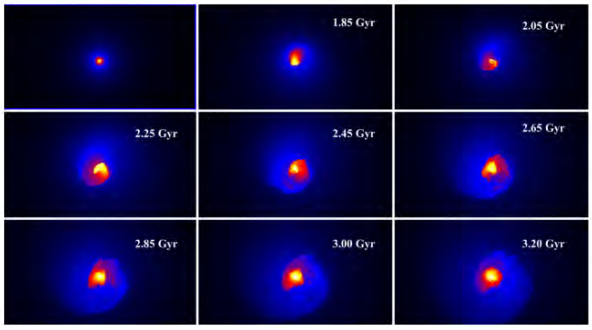

Figure 1. The evolution of a cold front from the simulations of sloshing in the magnetized cool gas in the cores of galaxy clusters for β = 200 [10].

bubbles were likely destroyed by the more developed side of the cold front, leaving the opposite bubble to move out from the center unhindered.

The galaxy clusters in Figures 3 and 4 match what is expected based on the simulations, as there are no bubbles outside of the cold fronts. Figure 5 shows what could be an exception. The Ophiuchus cluster has a curved bay feature approximately ~120 kpc southeast of the core center. For this feature to be a bubble, it would need to be incredibly powerful due to its massive size. We estimate the size of the potential bubble to have a radius of 155 kpc. As the bubble rises, it is expected to grow in size, which could explain why this potential bubble is as massive as it is. The bubble would also need to be very powerful to escape the cold front. However, we do not see its partner on the other side, nor does the GGM image elucidate a bubble. K-H instabilities, on the other hand, occur only on one side of the cluster. The Ophiuchus cluster has a low magnetic field strength which would make it susceptible to K-H instabilities. In addition, this bay feature is far from the center of the core, making it even more capable of producing K-H instabilities. Overall, it is more probable that it is not a bubble that causes this bay feature.

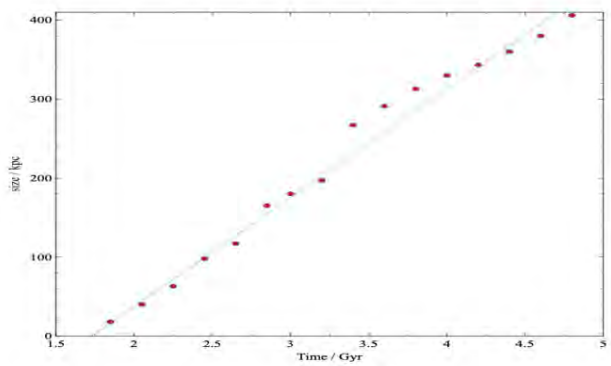

Figure 2. Plot of the growth of the cold front in regard to size and time.

Conclusion

We have investigated the cool-cores and X-ray cavities of Centaurus, Perseus, Ophiuchus, A2199, A262, A1795, 2A0335, A2029, and A85 galaxy clusters. All except Ophiuchus show bubbles that have not made it past the inner radius of the cold front. When comparing this with simulations of the interaction between rising bubbles and cold fronts in cool-core clusters, we find that this result is consistent.

The case of asymmetric bubbles appears to strengthen the argument, as it means that it is the cold front that disrupted and destroyed the missing bubble, making it unobservable. We also found that only the Perseus cluster had more than one pair of bubbles and that the 2A0335 cluster has two bubbles on one side. This furthers the evidence that the outer bubbles are getting distorted and destroyed by the cold fronts. It is possible that the outer bubbles exist but remain unseen due to the low photon counts at the outer edges of the clusters.

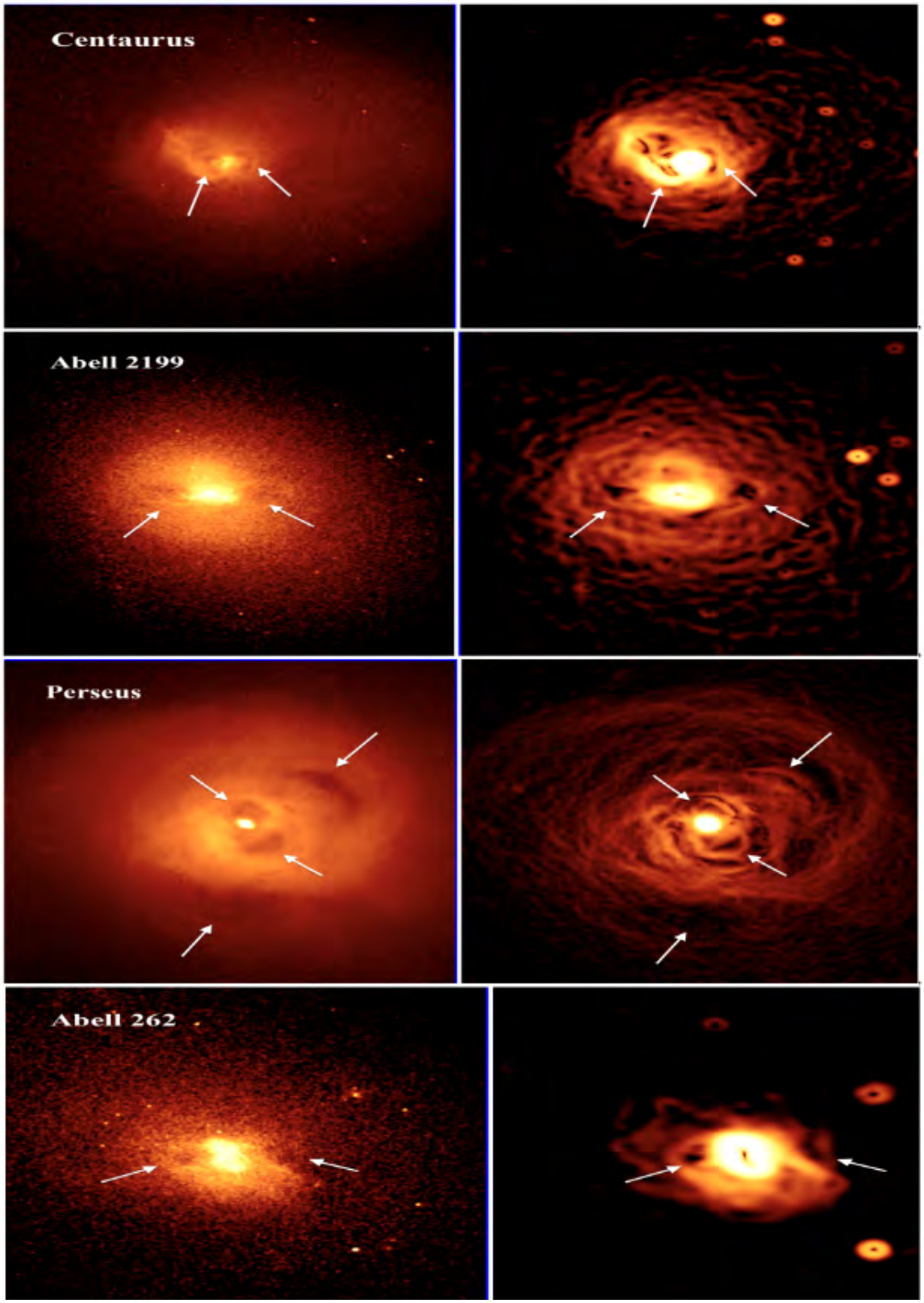

Figure 3. Group of galaxy clusters featuring bubbles on opposite sides of each other. The images on the right are the clusters filtered with the GGM filter to emphasize the bubbles and cold front structure.

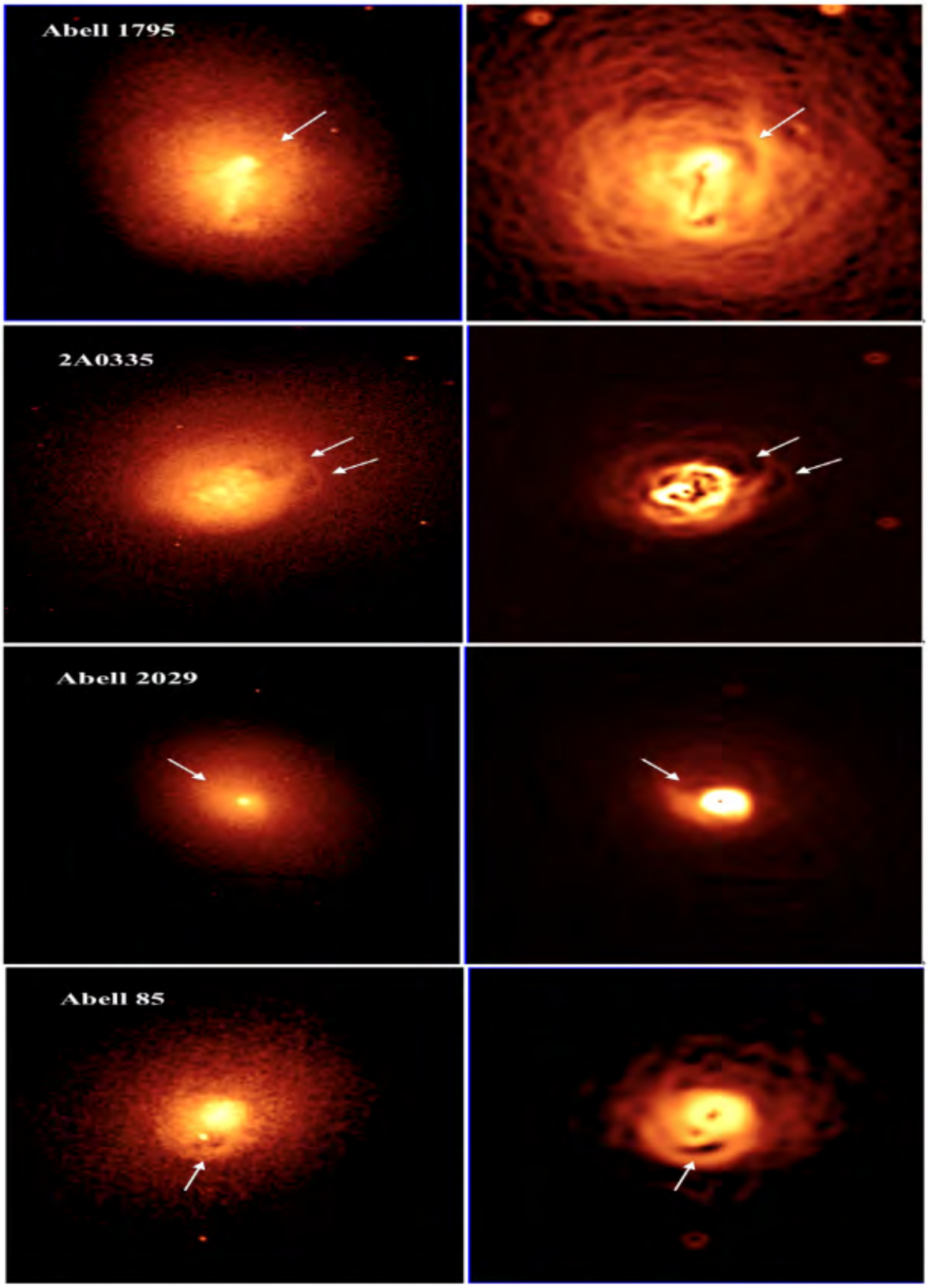

Figure 4. Group of galaxy clusters featuring bubbles on only one side. The images on the right are the clusters filtered with the GGM filter to emphasize the bubbles and cold front structure.

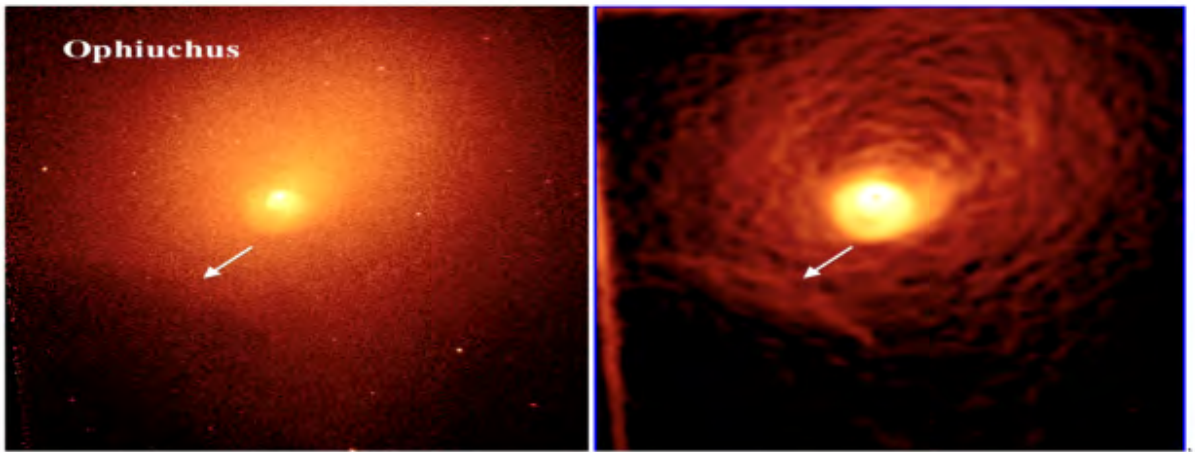

Figure 5. The Ophiuchus cluster featuring a potential bubble. The image on the right has been filtered with the GGM filter to emphasize the cold front and bubble structure. The GGM image is stacked from three images of σ = 8, 16, 32.

The conclusion made by S. Giacintucci supports the conclusion that the bay feature in Ophiuchus is not a bubble [6]. A detailed study of the cool core in Ophiuchus concluded that the southeast feature was probably caused by the gas dynamics from a merger [9]. However, from simulations of the sloshing of magnetized gas in cool-core clusters, we know that large bay features could be created by K-H instabilities in the sloshing cold fronts. Further analysis of the metal abundance, surface brightness, and temperature should be done on the bay feature in the Ophiuchus cluster to determine whether it is a K-H instability. Overall, the observational data aligns with the hypothesis that cold fronts are major disruptors to bubbles. In the future, a more systematic study needs to be done with a larger sample size to determine the prevalence of cold fronts that destroy bubbles.

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Appendix A: Tabulated Galaxy Cluster Information

Table A1. Calculated information for the galaxy clusters. For the bubble distance and cold front distance, the measurement is based on how far it is from the center of the core.

Galaxy Cluster	Speed of Sound (kpc/Gyr)	Bubble Distance (kpc)	Bubble Radius (kpc)	Cold Front Distance (kpc)
Ophiuchus	1570	120	155	42
Centaurus	1010	3.3 3.4	2.3 2.8	50
Perseus	1322	6.3 10.5 29 40	6.2 8.2 11.1 14.6	110
2A0335	861	28.1 32.5	7.8 3.5	53
A85	1333	18	7.5	82
A262	749	7 11	4.3 5.6	24
A1795	1417	19	11.5	85
A2029	1379	32	9.7	55
A2199	1033	20 21	12.2 12.6	60

Emotion Regulation Across Different Social Contexts

Alli Demoss[1], Dr. Katie Garrison[1]

Faculty Advisor: Dr. Katie Garrison[1]
[1]*Department of Psychology, The University of Alabama, Tuscaloosa, AL 35487*

The goal of this study was to understand how participants use two common emotion regulation strategies, cognitive reappraisal (CR) and expressive suppression (ES), in different social contexts. Additionally, we examined how individual differences in social anxiety moderates the effects of different social contexts on emotion regulation. Participants reported their use of CR and ES in response to hypothetical emotional situations, and we varied whether the situation occurred in the context of close others (family and friends) versus non-close others (new acquaintances). As expected, participants used more ES in negative situations as well as when among non-close others. Social anxiety (SA) predicted greater use of CR and ES, yet SA did not moderate the effects of social context or emotional valence on emotion regulation. Our hypotheses correctly predicted that social context would impact the use of ES, however, this effect was not amplified in those with higher SA as predicted.

Introduction

The significance of the following study is to understand the impact of social context on emotion regulation in individuals with various levels of social anxiety (SA). Emotion regulation is defined as the processes individuals use to modify the emotions they experience, the intensity of the emotion, the duration of the emotion, and the expression of emotion [5]. Emotion regulation is critical for maintaining a healthy social life and navigating emotional situations. This study highlights the importance of two distinct emotion regulation strategies: expressive suppression (ES) and cognitive reappraisal (CR). ES is a form of response modulation that is triggered when an individual inhibits their emotional expression to suppress what they are feeling. CR is the process by which an individual purposefully focuses on specific aspects of an emotional situation to re-frame its significance. The goal of the present research is to explore the extent to which individuals utilize ES and CR in response to emotional situations. Specifically, the study will assess the dependence of ES and CR on the particular social context surrounding the emotional event and individual differences in social anxiety (SA). There is a rising demand for research on emotion regulation involving social anxiety because Social Anxiety Disorder (SAD) is the fourth most common mental disorder in the United States [2]. Considering the frequency of SA, it is vital to gain an understanding of factors that influence emotional functioning in social anxiety [4].

Research suggests that SA is characterized by difficulties regulating emotions. The most common symptom of SA is feeling the need to avoid social events due to the fear of judgment from others [4]. Studies have found correlations between SA and emotion regulation, such that those with more SA demonstrated difficulties in emotional expressivity [10]. Other research has found that individuals with high levels of SA reported having difficulties with emotion regulation during social interactions in daily life compared to those with low SA [7]. Thus, the presence of SA may influence an individual's response to emotional situations.

SA appears to be linked to the use of ES and CR. Previous research supports the idea that individuals with SA use ES more than those without SA [5]. Additionally, when individuals use CR, those with SA demonstrate an inhibited ability to use this technique effectively [2]. Overall, most studies have concluded that individuals with SA tend to report using avoidant strategies like ES

more often than engagement strategies such as CR [1].

The social context might also influence how individuals regulate their emotions. Experiencing an emotional event in the presence of close others versus non-close others may shape individuals' choices about how they regulate their emotions. English et al. (2017) studied the factors predicting emotion regulation strategy use, such as the social context and participants' emotion regulation goals. The participants consisted of college students that completed daily diaries in which they reported instances where they used emotion regulation. The researchers found that the use of ES was higher when with non-close others, but CR did not depend on social context [3]. Thus, even individuals without SA may alter the ways they regulate their emotions based on the people who are in their natural environment.

Our study attempts to understand how individuals regulate their emotions during typical emotional situations, specifically focusing on ES and CR. Our research aims to explore how social context influences an individual's emotion regulation strategy and if SA further moderates this effect. We predict that the social context will influence emotion regulation, such that there will be less ES when among close others compared to non-close others. Predictions are less clear for how CR is influenced by social context. We also hypothesize that the valence of the emotional situation will influence emotion regulation, such that people will use more ES and CR in negative emotional situations compared to positive emotional situations. Lastly, we believe that the social context will have a stronger impact on ES and CR for those with higher social anxiety versus lower social anxiety.

Methods

Participants

Participants (N = 379) consisted of PY 101 students at the University of Alabama. The participants were compensated with credits that count towards their course.

Procedure and Design

Participants were randomly assigned to a social context (close others vs. non-close others), then presented with two positive and two negative hypothetical emotional scenarios in a random order that could occur in daily life (see Table 1). After reading each emotional scenario, participants were asked to imagine that they were experiencing the particular situation within their assigned social context, then were given a free response text box to report how they would feel in the situation. Next, the participants completed the main dependent measure assessing emotion regulation use, the Regulation of Emotion Systems-Ecological Momentary Assessment (RESS-EMA). This survey was developed specifically to measure emotion regulation strategy use in daily life and includes two items to assess the use of relaxation, engagement, rumination, reappraisal, distraction, and suppression (See Table 2) [9].

Finally, the participants filled out questionnaires assessing general social anxiety, including the Social Interaction Anxiety Scale (SIAS; [8]) and the Social Phobia Scale (SPS; [8]), emotion regulation tendencies (ERQ; [6]), and demographic information.

Data Analysis

The data analysis plan was pre-registered online (https://doi.org/10.17605/OSF.IO/TD23E). To test the first and second hypotheses, we ran a 2x2 mixed ANOVA using social context and emotional valence as factors. For the third hypothesis, we ran a multiple regression using factors of social anxiety, social context, emotional valence, and interactions involving social anxiety.

Our target sample size was 350 responses, which would give us 80% statistical power with an $a = 0.5$ to detect an effect size of $f = 0.13$. Data collection lasted the duration of a fall semester during the months of August through December.

Individuals that showed invariable responses across multiple scenarios, submitted nonsense or blank-free responses, did not follow instructions, or fall more than three standard deviations of the mean of the dependent measures were excluded from the data analysis.

Table 1. All emotional scenarios that were shown to participants followed by a free response answer space.

Emotional Scenarios

Positive	Negative
Date with Crush: You have had your eyes on a specific person for a few months now because they have a great personality and are very attractive to you. Y'all end up becoming close friends and get a text from them asking you out on a dinner date.	*Pet Died*: You have had your pet that you love dearly throughout your childhood. You have bonded with your pet throughout your life, but it has started aging, unfortunately. You receive a message from your vet saying your pet has passed away.
Receiving Honors:	*Failed Exam*:
You have been in school for many years figuring out what to do with your future and finally you find a specific subject that you are passionate about learning. After taking many classes and receiving high grades due to all your hard work, you are contacted by the university saying your hard work is recognized and you will be given honors.	It is your final exam and you have completed the study guide, discussed the content with your professor during office hours, and have been studying for hours all week so that you can pass the final exam. You take the exam and when you press submit, you receive a failing grade.

Participants under the age of 18 were also excluded. The final sample included 379 individuals. Data were analyzed both with and without exclusions to ensure our results did not depend on them.

Results

Effects of Social Context and Emotional Valence

We collapsed the two CR items (r = .455, r = .351) and the two ES items (r = .339, r = .230) within positive and negative scenarios, respectively. . The correlations were only moderate, but we proceeded to collapse them to analyze the results by valence.

Cognitive Reappraisal

The main effect of valence on CR was non-significant, F (1,377) = 0.196, p = .658, n_p^2 = .001. The interaction between valence and social context was non-significant, F (1, 377) = 0.810, p = .369, n_p^2 = .002, and the main effect of social context was non-significant, F (1, 377) = 0.734, p = .392, n_p^2 = .002. In summary, CR was not influenced by the valence or social context of the scenario (See Figure 1).

Expressive Suppression

A main effect of valence was observed on ES, F(1, 377) = 202.988, p <.001, n_p^2 = .350, such that ES was higher in the negative scenarios versus the positive scenarios (See Figure 2). We also observed a main effect of social context, F (1, 377) = 11.599, p = .001, n_p^2 = .030, such that ES was higher among non-close others versus close others. The interaction between valence and social context was non-significant, F(1, 377) = 2.142, = .144, n_p^2 = .006. Therefore the use of emotional suppression was influenced by both the emotional valence of the situation and the social context.

Table 2. *Regulation of Emotion Systems Survey – Ecological Momentary Assessment (RESS-EMA)* scale.

ER Strategy	Items
Relaxation	I would try to slow my heart rate and breathing I would take deep breaths
Engagement	I would show my feelings I would express my feelings
Rumination	I would think about the emotional event again and again I would continually think about what just happened
Reappraisal	I would think of other ways to interpret the situation I would look at the situation from several different angles
Distraction	I would engage in something else to keep busy I would engage in activities to distract myself
Suppression	I would make an effort to hide my feelings I would pretend I was not having feelings

Note: Medland, H., De France, K., Hellenstein, T., Mussoff, D., & Koval, P. (2020). Regulating emotion systems in everyday life: Reliability and validity of the RESS-EMA scale. European Journal of Psychological Assessment, 36, 437 – 446.

Interactions with Social Anxiety

The correlations between the SPS and SIAS were high (r =.76); therefore, we proceeded to average them into one variable, SA.

Cognitive Reappraisal

The analysis revealed that SA predicted greater use of CR, β = .252, SE = .112, t (359) = 2.26, p = .024. However, the Social Anxiety X Social Context interaction, β = -.013, SE = .112, t (359) = -.114, p = .909, the main effect of valence, β = -.067, SE = .073, t (1086) = -.923, p = .356, and the Social Context X Valence interaction, β = -.049, SE = .073, t (1086) = -.674, p = .500, were non-significant. Therefore, SA did not moderate the effects of social context or valence on CR, but social anxiety predicted greater use of CR.

Expressive Suppression

SA predicted greater use of ES, β = .526, SE = .107, t (359) = 4.930, p = <.001. However, the Social Anxiety X Social Context interaction, β = .117, SE = .107, t (359) = 1.100, p = .272, the main effect of valence, β = .046, SE = .080, t (1086) = .567, p = .571, and the Social Context X Valence interaction, β = .005, SE = .081, t (1086) = .063, p = .950, were all non-significant. Thus, SA predicted greater

suppression of emotion but did not moderate the effect of social context or valence on suppression.

Table 3

Descriptive Statistics

Emotional Scenario Averages	Mean	Std. Dev.
Crush CR	40.107	27.3306
Crush ES	27.516	26.3492
Honors CR	24.699	22.4883
Honors ES	16.334	21.4907
Pet CR	26.372	25.2094
Pet ES	34.726	31.0151
Exam CR	39.532	27.6736
Exam ES	47.449	31.2967

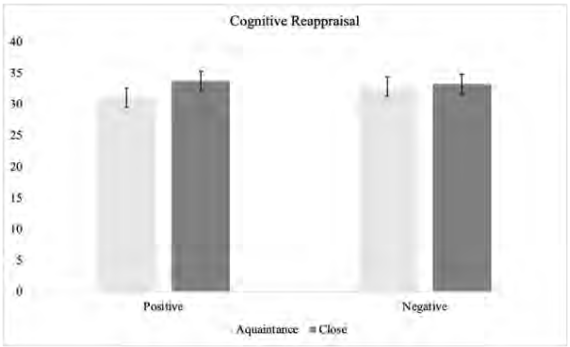

Figure 1. Cognitive reappraisal use as a function of emotion valence and social context.
Note: Error bars +/- 1 SE

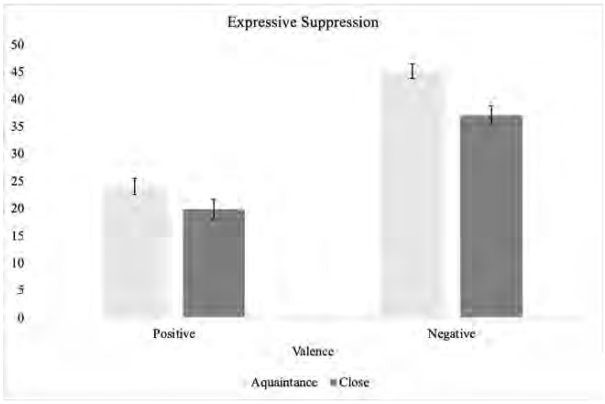

Figure 2. Expressive suppression use as a function of emotion valence and social context. *Note:* Error bars +/- 1 SE

Discussion

In this study, we addressed how undergraduate students use CR and ES based on emotionally charged situations and investigated if SA moderates the use of these emotion regulation strategies. We hypothesized that the use of ES would be used more significantly with non-close others rather than close others. The results supported this hypothesis, and we can infer that individuals are less likely to open up about emotional situations with people they are unfamiliar with compared to those they consider close friends or family. Furthermore, we predicted that participants would use more ES and CR in negative emotional situations relative to positive. The results partially supported our prediction–emotional valence had an effect on ES but not CR. These results indicate that individuals are more likely to suppress negative emotions relative to positive emotions, but cognitive reappraisal did not depend on the valence of the scenario.

Surprisingly, the results determined that individual differences in social anxiety did not amplify the use of emotion regulation strategies based on the social context of the scenario. Because SA is characterized by fearing social situations, it seemed logical that SA would moderate the effect of social contexts on emotion regulation, however that was not the case. This null finding could have been because the data were recorded via self-

report, which is a possible limitation. It would also be beneficial to expand research beyond undergraduate students to individuals of all age groups. Last, future research should test these effects in real-life emotional situations, rather than hypothetical scenarios. We designed the study to eliminate any bias or unrealistic responses so that the data is as accurate as possible. Further research is needed to help individuals experiencing social anxiety cope with emotional situations in different social contexts.

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The Unseen Families: A Systematic Review of Alternative Parental Interaction Techniques and Developmental Outcomes

E. Grace Turk[1], Dr. Evie A. Malaia[1]

Faculty Advisor: Dr. Evie A. Malaia[1]
[1]*Department of Communicative Disorders, The University of Alabama, Tuscaloosa, AL 35487*

Alternative parental interaction techniques are a familiar topic both in research and in clinical practice; parent coaching (or counseling) has long been a part of successful clinical interventions. However, even though parent coaching aims to change interaction techniques to those which are optimal for the parent-child dyad (e.g. more appropriate play support for a child with autism spectrum disorder), clinicians base their coaching on assuming static, typical models of interaction techniques. For this reason, it is critical to assemble the body of literature concerning parent interaction and analyze its strengths and weaknesses. The aim of this systematic review is to provide an analysis of the current literature on parent-child interaction and identify potential gaps that future research should address. Atypical parenting has the potential to add both depth and nuance to current models of parental interaction techniques, and it therefore deserves to be investigated in detail. However, despite its potential contribution to the understanding of child development, articles objectively and critically measuring atypical parenting are scarce and typically qualitative. Atypical parents and their interaction techniques ought to be identified and explored in detail for the benefit of academics and clinicians alike, as examining atypical parents who produce positive developmental outcomes has the potential to greatly enrich current models of childhood development.

Introduction

The most recent statistic from the Center for Disease Control and Prevention reports that, as of 2020, 26% of American adults live with disabilities [9]. The fundamental definition of a disability is any characteristic that changes the way a person interacts with the world around them [9]. These facts together indicate that one out of every four American adults interact with the world around them in a way that is atypical when compared to an abled norm.

Approximately 83% of disabled Americans will have children [4]. Disabled parents therefore make up 23% of all American parents and often will parent atypically as a result of their disability. Even though typical parenthood has been considered in numerous research studies, these atypical techniques still remain thoroughly underinvestigated. In order to fully understand potential trajectories in child development, parent-child interaction, and disabilities as a whole, atypical parenting techniques must also be considered.

At present, parent-child interaction techniques that yield typically developing children are assumed to be guided by abled parenthood, and the intersection of disability and parenthood appears only to be included in research when the developmental outcomes it produces are below the norm. However, it is impossible that disabled parenthood unilaterally produces children with atypical, disadvantageous development. Conducting research with this population has the potential to contribute significantly to both literature and clinical culture. This research would additionally provide this population with visibility and acknowledgement. For these reasons, the following review was conducted to assess the completeness of the literature regarding parent-child interaction techniques.

Methods

Systematic Review Protocol

In compliance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis, a search of the literature was

conducted, defining eligibility according to the PICOS model of populations, interventions, comparators, outcomes, and study design [36].

The population reviewed in this study was defined according to its performance of or exposure to parental interaction techniques. Initially, the population was limited to familial households parenting typical children wherein one or more parents was hard-of-hearing with low support needs. This search query produced one result: an article authored by Mitchell and Karchmer, whose primary focus was on deaf rather than hard-of-hearing parents [27]. The search was then expanded to review all disabled parents, but the results lacked scale and diversity. The final population definition included families with disabled parents, abled parents, typical parents exposed to parent-coaching, or typical parents not exposed to parent-coaching.

Though some populations reviewed by this study focused on the subject of parent-coaching, no single intervention method was common across the literature reviewed. Specific intervention was thus omitted from the search query, as it would not have added any particular value.

In the experiments reviewed, children who received alternative parental interaction techniques were compared to established norms or control groups, so the addition of terms for comparison would not have added value to the search.

Outcomes across the reviewed studies varied on a spectrum of negative to positive child development. Any evidence of the effect of alternative parental interaction technique upon child development qualified studies to be included in the review. Children and child development was thus included in the search.

As the intent of the review was to assess parental interaction techniques and their effect on developmental outcomes, any study designed which measured this relationship in some way was considered applicable. Study design was therefore not included in the search query. Review articles were included, which enabled other researchers' assessments of the literature to guide this study's discussion of the literature. Only records written in English were included.

Data Sources and Search Strategy

In initial searches, Web of Science, JSTOR, APA PsycInfo and PubMed databases were used. The queries contained keywords which limited results to hard-of-hearing parents and their typically developing children. Results were not filtered by date of publication. The initial search was conducted on January 21st, 2022. It yielded only one relevant result: an article written by Mitchell and Karchmer in 2004 [27]. The same search was run again in February, but no additional articles were found. In March, the search was expanded to include articles focusing on all atypical parenting styles, and the initial variety of databases eventually was narrowed to focus on PubMed, as results became more readily available under the umbrella of a broader search.

Study Selection and Data Extraction

At the identification level, the search results were screened by information found in the title and abstract. Once this initial identification was performed, the full texts were reviewed. Though backward citation search was performed specifically for the article written by Mitchell and Karchmer, it yielded no relevant articles and therefore did not contribute to the body of research presented in this systematic review [27]. The studies selected from the search performed in March were chosen based on how succinctly and specifically they summarized varying aspects of parent interaction and child development, or how they could contribute to a general understanding of the topic and provide background detail.

The variables this review considered relevant were (1) natural parental interaction techniques, (2) clinical intervention, and (3) positive or negative developmental outcomes in children studied. The first term, "natural parental interaction techniques," refers to techniques uninfluenced by clinical or research-based intervention, though these techniques may be influenced by other factors, such as disability. This category was later separated into natural abled parental interaction and natural disabled parental interaction. The second term, "clinical intervention," refers to techniques such as parent-coaching, wherein a clinician or researcher prescribes specific kinds of responses

for the parent to make in response to their child. The final term, "developmental outcomes," were reported by a wide variety of measures, including educational attainment, language development, and emotional health. Intentional ambiguity has been included in the language of this review in order to emphasize that the specific developmental measure is highly variable, and therefore can only be reported as net positive or negative.

Study Selection and Characteristics

As previously mentioned, the initial searches conducted in January and February of 2022 yielded only one result relevant to the query: Mitchell and Karchmer [27]. This query specified that the population must be hearing families with one hard-of-hearing parent, and that the developmental measure must be articulation development in childhood speech. This search query was discarded in early March, on the basis that the literature was too sparse to warrant a systematic review. Although excluded from this systematic review, future studies could expand on the original research query.

Once the search query was rewritten to broaden the topic, 3,002 results were obtained from PubMed. Seventy-four of these records were subsequently screened, and those which did not meet the aforementioned criteria excluded from consideration (see PRISMA flow diagram). It was not necessary to remove any duplicates. Forty records were excluded, and the remaining 34 were assessed for eligibility. During a review of the full text, a further 2 records were excluded on the basis of inclusion criteria. The final number of full text studies reviewed was 31, with only one subsequent report.

Results

Childhood development is a multifaceted topic with a myriad of measures, especially in terms of outcomes. It is for this reason that the following ambiguous terms will be used in the synthesis to refer to the body of literature. "Positive" will refer to childhood outcomes that are desirable either to the parent or to the society in which the child lives. "Negative" will refer to childhood outcomes that are undesirable to the parent and a hindrance to the child societally.

Studies which examine abled, typical, natural parent interaction techniques tend to agree that outcomes are, as a whole, positive. These studies include entries 1, 2, and 7 from Table 1. For example, Dave, Mastergeorge, and Olswang [13] published a study of the effects of motherese and affect on vocabulary development and dyadic communication in infants and toddlers. In this study, mother–infant unstructured interactions were recorded at 7 months and 11 months. Dave et al. subsequently scored these participants for "(i) maternal use of guiding speech and (ii) maternal dominant affect". In order to determine the effect of these two variables, participants were invited back at 18 and 24 months in order to report child language performance via the MacArthur-Bates Communicative Development Inventories [16]. The results indicated that particular elements of maternal communication affected a few different aspects of developmental outcomes, and that no maternal communication elements overtly hindered or harmed childhood language development.

Swanson et al. [39] performed a similar study in 2019 reviewing household language exposure for infants at risk for autism. This study found that environments more rich with language tended to be more beneficial to language development, but did not report any outcomes attained as necessarily deleterious to any child in any environment. Though results vary in measure, no specific, abled, natural parent interaction appears to yield a negative outcome, providing a clear example of how this kind of interaction technique tends to be reported positively in research studies.

Studies which examine abled, typical, clinical parent interaction techniques follow a similar pattern, as clinicians and researchers alike are ethically prohibited from providing intervention that may overtly harm a child. These studies include entries 3, 5-6, and 8-9 from Table 1. For example, Ferjan Ramírez, Lytle, and Kuhl in 2019 conducted a study which compared control groups of typical parents and children to experimental groups of clinically coached parents and children and found that, "Intervention infants showed greater

growth in babbling. . . [and] produced significantly more words than Control infants" [17].

In this same category, Whitehouse and a team of 35 researchers conducted a randomized control trial over the course of four years with 104 infants aged 9 to 14 months, who showed early behaviors associated with autism spectrum disorder (ASD). The selected participants were randomized on a 1:1 ratio of receiving preemptive intervention and usual care or only receiving usual care over a five month period. Preemptive intervention, in this case, was defined as a 10-session iBASIS-Video Interaction to Promote Positive Parenting method–a type of social communication intervention based on Applied Behavioural Analysis [40, 43]. In the iBASIS-VIPP group, only 6.7% were classified as having ASD, while in the usual care group 20.5% of the infants were classified as having ASD. The results of the study demonstrated that preemptive ASD intervention led to a decrease in significant severity of symptoms in early childhood and mitigated the child's chances of being diagnosed with ASD later on [43]. In examples of abled, typical parenting, it is evident that research categorically demonstrates positive developmental outcomes.

Studies which examine disabled, atypical, natural parent interactions tend to report that children of disabled, atypical parents attain primarily negative outcomes, whether these outcomes be educational, emotional, or developmental. These studies include entries 10-17 in Table 1. For example, LeClere and Kowalewski [22] published a multi-variable study on the effect of familial disability on the well-being of children. Their overall message was best exemplified in one notable statistic they reported; children who live in a household with one or more disabled people had an adjusted mean index of the BPI-I (Behaviour Problems Inventory) over 50% larger than their typically situated counterparts, indicating behavioral issues worsen in disabled households. These children were attaining what this review would label a negative developmental outcome.

In another example of this type of study, Aoyagi and Tsuchiya [5] examined the literature regarding the effects of maternal postpartum depression on the four key areas of child development: physical, neuromotor, language, and general cognitive. Although lacking wide-scale, longitudinal studies concerning their topic, they found a few notable studies which drew correlations between postpartum depression (PPD) and stunted physical development in developed countries, as well as a slight delay in fine motor and language development. They strongly recommended further research on the topic in order to confirm or negate these results.

Shandra and Hogan [38] also assert that the presence of disability in a family lowers parental expectations for educational attainment, which leads to lower educational attainment. Parents' diminished expectations of children's potential for education is negatively associated with the child's chance of attaining a high school diploma. However, Shandra and Hogan are careful to note that, in cases of disabled children themselves, "disability does not significantly reduce children's own expectations after accounting for school performance". The child's educational attainment relies heavily on the expectation of the parent.

Overall, the presence of disability in the family is widely regarded as a negative influence that produces negative developmental outcomes. Even in studies where the authors are careful to perform inclusive analysis of the literature and assert that more research should be done to substantiate these claims, bias against families with disabilities is strong and pervasive [28, 33].

Discussion

The purpose of this review was to assess the integrity and scope of the literature regarding parental interaction techniques in order to accurately interpret whether current clinical models fully regard all modes of interaction and their impact on childhood developmental outcomes. Given the rising interest in parent-coaching as a clinical practice, it is important that such a multifaceted topic be thoroughly fleshed out in research. While it seems as though the literature surrounding this topic thoroughly explores both harmful and helpful practice, important 'gaps' exist that must be addressed concerning disabled parenting and developmental outcomes. These 'gaps' expose not only a heretofore unresearched subject area,

but a predisposition of clinical research to assume disabled parenting will always neglect the child in some specific area.

The thirty-one studies included in this review present both varying experimental designs and result formatting. Developmental outcomes have never been, and never will be, a perfectly objective unit of measurement. However, in an attempt to present as holistic a view as possible, articles concerning various outcomes were included (i.e. emotional and educational attainment, language milestones, social development, and cognitive outcomes). Though such a wide net was cast in terms of inclusion criteria, the results of the review show that a substantial gap exists in current research models, specifically regarding disabled parents with low support needs.

Nature's Scaffolding

Studies of natural parent-child interaction techniques and their effects on development outcomes, while not as prevalent as their counterparts regarding clinical interaction, substantiate a major category of research. As a whole, these articles tend to assess natural parent deficiency or sufficiency and seldom make any mention of disability at all, except in cases such as Mitchell and Karchmer [4, 13, 18, 26]. All articles in this category make strong recommendations for further research on the basis of scarcity of literature.

In a systematic review of the literature regarding maternal postpartum depression and developmental outcomes, Aoyagi and Tsuchiya found that there appears to be a common association between PPD and the four areas of child development: physical, neuromotor, language and general cognitive. Their review, however, clearly demonstrated a lack of "large-scale, longitudinal studies with a long term follow-up" [5].

A later study by Kucker et al. [21] yielded conclusive results regarding the link between three positive parenting traits and childhood language development. Parent openness, consciousness, and agreeableness "appear[ed] to impact a child's language abilities above and beyond well-known predictors of language,". However, the rationale provided by the authors of this study asserted that, to their knowledge, "no work has examined how parent personality impacts language development". The results of this particular experiment, if true, could have substantial impact on the future of parent-coaching in clinical settings, and therefore further research is not only warranted, but critical.

Clinical Scaffolding

Parent coaching, especially during the COVID-19 pandemic, has become a widespread technique used by a variety of clinicians across the globe. Traditionally, parents are trained in clinical techniques that have thus far been proven effective in therapeutic settings. Most research surrounding this topic focuses on the quality of parent interaction, as most parents naturally interact with their child an appropriate amount. Studies in this domain conclude that providing feedback to the parent regarding their own interaction with their child often improves the language environment of the child and, in turn, improves early learning milestones.

Two studies in the category of clinical parent coaching were conducted by Ferjan Ramírez, Lytle, and Kuhl in 2019 and 2020 [16, 17]. Their research, though both well-designed and thoroughly documented, has a critical pitfall of not following participants past two years of age. Ferjan Ramírez et al. [17] utilized Language Environment Analysis (LENA) recordings to ascertain the progress of the children and supplemented these recordings with MacArthur-Bates Communicative Developmental Inventory, a survey given to parents [16]. Its conclusion, though highly generalized, was that parent coaching "can immediately and positively impact child language outcomes" [17].

In a follow-up study, performed in 2020, three hypotheses were presented by the researchers. Those hypotheses are as follows:

> *"The present study was designed to test three specific hypotheses: That a parent coaching intervention delivered at 6, 10, and 14 mo would: 1) Increase parental use of parentese speech, but not parental use of standard speech, or the overall quantity of parental speech; 2) increase parent–child turn-taking between 6 and 18 mo; and 3) lead to*

> *enhanced growth in child vocalizations during the same time period, and enhanced language outcomes at 18 mo, 4 mo after the third coaching appointment"* [18].

The results of the study conclusively support all three hypotheses, proving parent coaching to be an effective tool in the clinician's toolbox.

Parent coaching is effective in parenting typical and atypical children alike. Across the spectrum of ability, studies confirm that clinical intervention in parental interaction techniques can be highly beneficial.

One such study that was published by Swanson et al. [39] and dealt with infants at risk for autism, assessed the effects of early language exposure on language skills of infants with and without autism. The researchers found that, "parent behavior during the earliest stages of life can have a significant impact on later development". This study contained three groups of subjects across the spectrum of infantile ability: "high familial-risk infants who did not have ASD. . . high-familial-risk infants who had ASD. . . and low-familial-risk infants who exhibited typical development".

All infants in the above study were assessed through two all-day home recordings, and the data conclusively showed that exposure to adult language and more conversational turns were eventually associated with improved language skills. However, even in Swanson et al.'s well-designed, thoroughly documented study, we find another limitation in that language skills are not measured into later childhood. This study also stopped at 24 months, only extending six months past the final survey of Ferjan Ramírez's study in 2020 [38, 17].

Young Carers

"Young Carers" are children of disabled parents who take upon themselves an excessive burden of care, often to their own personal detriment. Articles in this category tend to emphasize that having a disabled parent yields negative developmental outcomes.

In the early 2000's, there was a string of articles following the publication of a book called Children Who Care: Inside the World of Young Carers. The release of this book from Aldridge and Becker in 1993 seems to have sparked a new kind of discussion regarding young carers and the support they receive from society; the new discussion is primarily political in nature, rather than developmental, as all parties agree that more socialized support for disabled parents would reduce the likelihood that their children display negative developmental outcomes.

First, in 1994, an article was published by LeClere and Kowalewski that determinedly asserted the negative outcomes children of disabled parents displayed. Not only did this study thoroughly document the increased risk of behavioral issues as measured by the Behavioral Problems Inventory I (BPI-I) and the Behavioral Problems Inventory II (BPI-II), it also took into account what having more than one disabled family member meant for developmental outcomes. Children in a home with more than one disabled family member were not only found to be the most likely to show significant behavioral issues, they were also shown to be at increased risk of accident, injury, or poisoning [22].

Later, in 2002, Newman authored an article entitled, "'Young Carers' and Disabled Parents: Time for a Change of Direction?" wherein he asserted a very similar premise to that of Children Who Care. He argues that though strides have been made in research and policy to assist children of disabled families who are displaying negative outcomes, more work needs to be done to assess current systems and provide caretaking support [30].

More recently, in 2009, Rivera Drew [37] authored an article which spurred intense discussion regarding the validity of previously published literature, its lack of control groups, and its omission of cultural and social considerations. Her perspective is possibly the most well-researched of the previously mentioned articles, as she investigates the foundational question of this review: how can we possibly expect to group all disabled parents into one category? She concludes her paper with the following exhortation:

> *"[W]e must continue to pursue research providing critical insight into the development of a fairer standard of parental*

> *capacity that both acknowledges that successful parenting often includes reliance on paid and unpaid helpers outside of the nuclear family, and that minimizes the effects of prejudicial attitudes towards parents with disabilities" [37].*

The Gap

See Table 3

The title of this systematic review references the unseen families, those whose parental ability is not negatively affected by their disability. These parents' disability changes their parenting style without preventing them from fulfilling parental responsibilities. As stated in the introduction, currently up to 23% of parents in the United States have a disability, but we see the research has stalled, with the most comprehensive surveys found by this review occurring in the UK between 1990 and 2010.

The lack of inclusive research surrounding disabled parenthood only serves to confirm the bias that disabled parents are somehow deficient in their parenting capacity and require clinical support in order to be successful. With the current rates of disability and parenthood in the United States, this simply cannot be a universal rule.

It is therefore evident that a gap in the current literature both exists and actively creates a blind spot in the literature that subsequently confirms biases and prejudicial attitudes about disabled parenthood. It is critically important that this gap be filled if clinicians and researchers alike are to expand their methodology and accept neurodiversity in its wholeness. Not all disabled parents are cases that require clinical intervention, and to assume so is to expose an inherent bias against not only disabled parents, but disability as a whole.

Recommendations for Future Research

Long-term, wide-scale, critically designed studies of families with disabilities represent the first step towards achieving the goal of neurodiverse acceptance and dignity. These families must first be identified so that a population may be established for researchers to compare to typical controls. They must subsequently be surveyed and followed over a long period of time in order to establish their patterns of behavior and parenting. Then, they must be compared to their typical counterparts in order to see what aspects of parenting they have in common, what they do differently, and which method holds better results and potentially clinical significance.

Leaving these families unseen significantly hinders the perspective of clinicians and researchers. If, when searching for resources on how to counsel a disabled parent, clinicians only have access to 10-30 year old articles that insist behavioral issues are caused by disabled parenthood, clinicians will develop a negative attitude towards disability, and disabled parents will be discouraged.

With the advent of the neurodiversity movement and the importance of accepting atypicalities in society, it is critical that this future work be done. Though these families exist, thus far they have been overlooked because their children develop typically and do not require clinical solutions. However, a well-rounded perspective of parenting across the spectrum of ability in research and in clinical practice is a necessity as discourse and literature progress towards inclusion.

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Appendix

Table 1. An overview of papers reviewed by citation, population, variable, and result organized by the factors of ability, clinical intervention, and developmental outcomes documented therein.

Title	1. Taking Parent Personality and Child Temperament into Account in Child Language Development	2. Emotional and Behavioral Characteristics of Gifted Children and Their Families	3. Effect of Preemptive Intervention on Developmental Outcomes Among Infants Showing Early Signs of Autism	4. Early Language Exposure Supports Later Language Skills in Infants with and without Autism	5. An Evaluation of a Behavioral
Citation	Kucker et al.	Eren et al.	Whitehouse et al.	Swanson et al.	Morawaska and Sanders
Population	Early Childhood Development	Early Childhood Development	Early Childhood Development	Early Childhood Development	Early Childhood Development
Variable	Parent and Child Personality	Child Ability	Interaction Style, Child Ability	Interaction Style, Child Ability/Disability	Interaction Style, Child Ability
Result	Background	Background	Positive	Positive	Positive

6. A Study of Assisted Problem-Solving	7. Motherese, Affect, and Vocabulary Development	8. Parent Coaching Increases Conversational Turns and Advances Infant Language Development	9. Parent Coaching at 6 and 10 Months Improves Language Outcomes at 14 Months	10. Does Maternal Postpartum Depression Affect Children's Developmental Outcomes?	11. Disability in the Family: The Effects on Children's Well-Being
Wood and Middleton	Dave, et al.	Ferjan Ramirez et al.	Ferjan Ramirez et al.	Aoyagi and Tsuchiya	LeClere and Kowalski
Early Childhood Development	Early Childhood Development	Early Childhood Development	Early Childhood Development	Atypical Parents, Early Childhood Development	Atypical Parents, Early Childhood Development
Interaction Style	Interaction Style	Interaction Style	Interaction Style	Parental Disability	Parental /Disability
Background	Positive	Positive	Positive	Negative	Negative

12. When Parents are Deaf versus Hard of Hearing	13. Children Who Care: Inside the World of Young Carers	14. Does Maternal Postpartum Depression Affect Children's Developmental Outcomes?	15. Disability in the Family: The Effects on Children's Well-Being	16. When Parents are Deaf versus Hard of Hearing	17. Children Who Care: Inside the World of Young Carers
Mitchell and Karchmer	Aldridge and Becker	Monroe and de Andrade	Newman	Rivera Drew	Shandra and Hogan
Atypical Parents, Early Childhood Development	Atypical Parents, Childhood Development	Atypical Parents, Childhood Development	Atypical Parents, Childhood Development	Atypical Parents, Childhood Development	Atypical Parents, Childhood Development
Parental Disability	Parental Disability	Parental Disability	Parental Disability	Parental Disability	Parental Disability
Negative	Negative	Negative	Negative	Negative	Negative

Table 2. Diagram of the methodology for identification of included studies.

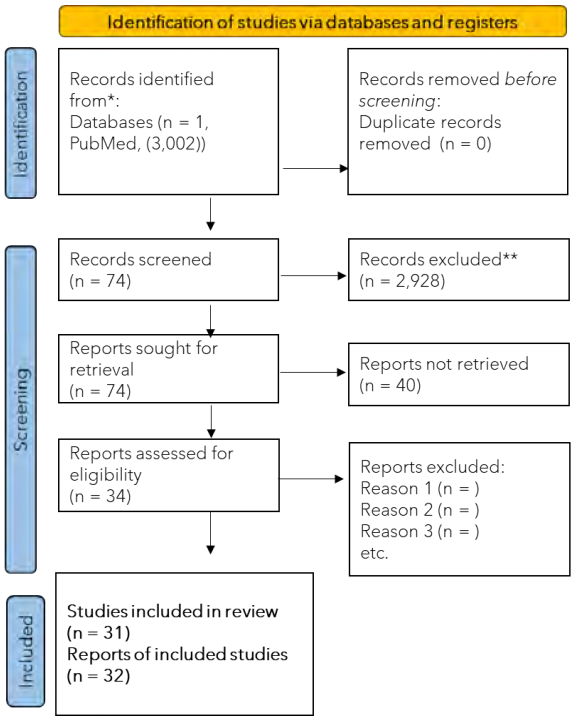

Table 3. A visual representation of the interaction between and results of the four factors analyzed in this review.

		Outcomes: Negative	Outcomes: Positive
Parent Ability	Abled	(IMPLIED): Lack of parent coaching yields suboptimal outcomes e.g.: improper handling of disability and neurodiversity, etc.	Parent coaching yields optimal outcomes; parent behaviors positively influence child development
	Disabled	Disabled parenting overburdens children and yields negative educational and social outcomes	No Studies Found

Recent Developments in Spinning Starch Fibers by Electrospinning

Richard LaLance[1], Dr. Lingyan Kong[1]

Faculty Advisor: Dr. Lingyan Kong[1]
[1]*Department of Human Nutrition and Hospitality Management, The University of Alabama, Tuscaloosa, AL 35487*

Starch shows great potential for the fabrication of nanofibers by electrospinning, as it is an inexpensive and widely available starting material. However, there are challenges to electrospinning pure starch nanofibers that require exploration. Electrospinning is a technique that has been used to fabricate biopolymer nanofibers for research and industrial purposes. This technique uses a high voltage power supply, a syringe pump, a syringe, a spinneret, and a grounded collector. A polymer dispersion, or dope, is pushed through the syringe, and the applied electrical field breaks the surface tension and creates a jet that ultimately becomes the nanofiber. The three main challenges for the fabrication of pure starch nanofibers are breaking down starch from its natural granule form, understanding the effect of different starch compositions, and optimizing the electrospinning parameters and setup for optimal fiber characteristics. This review investigates recent efforts in overcoming these challenges to fabricate starch fibers by electrospinning.

Introduction

Recent decades have seen a great interest in producing fibers from biopolymers such as starch [1]. Starch is an inexpensive and abundant starting material. Therefore, it is ideal to find ways to fabricate pure starch fibers with desirable mechanical properties and functionalities on a micro- and nano-scale. Additionally, achieving such fibers is important for understanding the mechanism behind the electrospinning of biopolymers. This understanding can open the door for the development of other functional biomaterials.

Three major challenges become prevalent in the electrospinning of pure starch fibers, and these must be addressed. Starch in its native state exists as granules with a complex hierarchical structure, ranging from linear and branched components to helices, random chains, and crystalline and amorphous lamellae [2]. Typically, breaking down granules to random coils is necessary in order to attempt electrospinning, but at ambient temperature starch granules are not soluble in water or many other organic solvents. Therefore, finding proper dissolution techniques is required. Additionally, the composition of the starch is important in solvent selection and in determining electrospinning parameters. Specifically, the content of amylose, amylopectin, and their respective molecular characteristics' roles in electrospinning are of interest. Lastly, the setup of the electrospinning device and its optional parameters must be optimized to obtain fibers of desired morphology and properties. Previous research has made great progress toward solving these challenges, but much work is yet to be done.

Breaking Down Native Starch

In general, there are three ways to completely break down starch into random coils or loose molecules. The first is the high temperature method, which heats amylose to temperatures greater than 170 °C and treats with liquid water [3]. The temperature required is higher than the gelatinization temperature of starch and is regarded as the "clearing point" during helix-to-coil transition. The second method is the dimethyl sulfoxide (DMSO) method [4]. In this method, starch is dissolved in pure DMSO or an aqueous DMSO solution at a temperature below its boiling point. The third method is the alkali method, which uses sodium hydroxide or potassium hydroxide (e.g., 0.01-0.1 M) to dissolve starch [5]. Ionic liquids, organic

salts with melting points below 100 °C, were explored as other possible solvents [6]. While they show potential, ionic liquids are expensive and difficult to remove during the electrospinning process.

To prepare the electrospinning dope, or biopolymer dispersion, there are two established methods for dissolving pure natural starch, and these are the DMSO system and the formic acid system [7-9]. Both solvents lead to a clear dispersion with shear thinning behavior. Depending on the method used, the fiber morphology and diameter distribution range from hundreds of nanometers to a few microns.

DMSO is capable of breaking down starch granules to allow for the electrospinning of pure starch fibers. The DMSO method utilizes wet-electrospinning, as DMSO is a nonvolatile solvent. DMSO is diffused into a nonsolvent bath such as ethanol or acetone. In this method, the starch polymer chains' hydrogen bonds are dissociated by aprotic DMSO, a strong hydrogen bond acceptor, and this forms random coils. When studying the effect of DMSO/water solvents on starch granule dissolution, the spinnability of starch dispersions, and the properties of electrospun starch fibers, researchers discovered that >85% (v/v) DMSO is required to make fluid-like starch dispersion for adequate electrospinning capability, and low concentration starch dispersions (5% w/v) are incapable of forming fibers [10].

Another method for electrospinning pure starch fibers following the breakdown of starch granules is the formic acid method. In contrast to using DMSO, the formic acid-dissolved starch is appropriate for dry-electrospinning, as formic acid is a volatile solvent. In this method, starch undergoes o-formylation, which is rapid esterification. This allows for the creation of starch-formate fibers. Over 70% (w/v) formic acid is required to completely disentangle amylose [9].

The results of using DMSO and formic acid to electrospin pure starch nanofibers can be understood through scaling theory. This method is often used to test rheological properties instead of the analysis of amylose content. It can be used to understand the degree of entanglement and characterize electrospinning dopes [11]. This is because both solvent and polymer characteristics are responsible for a dispersion's polymer entanglement and rheological properties. This method uses the relationship between concentration and specific viscosity to determine four concentration regimes: dilute, semidilute unentangled, semidilute entangled, and concentrated. These regimes are determined by plotting specific viscosity against concentration. The scaling number is the dependence of the specific viscosity on the polymer concentration in each regime. The entanglement concentration is the critical concentration which separates the semidilute unentangled regime from the semidilute entangled regime [12]. Certain concentrations above the entanglement concentration have been found effective in electrospinning. For example, high amylose maize starch in DMSO required 1.2-2.7 times the entanglement concentration [7], and amylose solutions in formic acid used 1.6 times the entanglement concentration [13].

Many imperfections have been discovered in the current methods used for electrospinning pure natural starch fibers, allowing for new exploration and improvement. Both established methods of electrospinning these fibers include the use of potentially problematic organic solvents. These organic solvents can be toxic to humans, and dealing with large amounts of chemical waste could deter the use of such solvents in commercial electrospinning applications. In addition, DMSO and formic acid may leave residue on the fibers. This can limit their application in industries such as food. In the DMSO method, the use of wet-electrospinning requires a long drying step, which can be greater than six hours. As well, the formic acid method causes the starch to age over time, and this causes the results of electrospinning for identical dispersions to vary over time [9]. Overall, while current methods show potential, the process of electrospinning pure natural starch fibers requires improvement in order to be ecologically sustainable, commercially efficient, and consistently safe.

Starch Composition

The composition of pure starch presents another challenge to the electrospinning of these compounds into fibers. When considering

natural starch, its amylose content, polymer chain structure, and molecular weight distribution all vary. Moreover, these characteristics are highly responsible for properties of the spinning dope such as viscosity, surface tension, and molecular entanglement. Specifically, the ratio of amylose to amylopectin is very important in regards to the spinnability of a starch dispersion, and continuous starch fibers are generally fabricated from amylose or high amylose starches. Dispersions containing less than 25% amylose content have been found incapable of being electrospun into fibers [7]. Some trials using starch dispersions with less than 50% amylose content resulted in beady, ribbon-shaped fibers. Chain entanglement is required for the formation of continuous fibers, and this is made possible by amylose's linear structure. Amylopectin does not assist in chain entanglement, as it forms globular structures, or gel-balls, in proper solvents. Despite this, research has shown that there may be potential for effectively electrospinning fibers with higher amylopectin content.

Recently, different mixtures of formylated amylose and amylopectin from formic acid have been the topic of electrospinning research, and the previously mentioned adverse effect of amylopectin on the ability to undergo electrospinning has been evaluated [13]. The influence of the amylose to amylopectin ratio on rheological behavior and spinnability of dispersions and the effect of aging on the mechanical properties of electrospun fibers were demonstrated as follows. This study used a polymer concentration of 17% (w/v) to study aging behavior in formic acid. While amylopectin in concentrations above 10% (w/v) has limited solubility in formic acid, amylopectin solution becomes transparent and less viscous due to aging. This is a result of acid hydrolysis which leads to lower polymer molecular weight as time progresses. Because of the necessity for amylopectin to age, the solution needed 2.5 days of aging in order to undergo electrospinning. In contrast, amylose was ready in several hours, but due to hydrolysis it lost spinnability after 1-1.5 days in storage. The two different dispersion types lead to the fabrication of fibers with differing morphology. Fibers from amylopectin formed short, wrinkled, beady fibers, and fibers from amylose were uniform. Mixtures of amylose and amylopectin were prepared by timing the dissolution carefully. The amylopectin was made first, and amylose was added after two days of aging. From there, the dispersions were aged for 12 hours, so amylose could become optimally spinnable. Researchers claimed that amylose and amylopectin are incompatible in formic acid although no segregation between the two were observed in the dispersions. Amylose presence had a positive correlation to the fiber morphology, but the ratio of amylose to amylopectin was not found to have a substantial impact. The mechanical strength and elongation at break of the fiber mats increased with increasing amylose. This study demonstrated the importance of amylose in the electrospinning of starch fibers.

The challenge posed by the composition of starch on the electrospinning of pure starch fibers makes it important to look at different sources of pure, natural starches, as they are each inherently unique in their composition. Previous research has led to studies that look at aging starch with different starch types, solution properties, and fiber characterization [9, 14, 15]. After aging, potato starch displayed Newtonian behavior was, and hydrolysis from aging helped deconstruct starch polymers and reduce the viscosity of the dispersion [14]. At the same time, starch from maize displayed shear thinning behavior [9, 13]. The observed fiber diameter had a positive correlation with viscosity, with diameter decreasing along with viscosity [9, 14, 15]. Furthermore, similar to the DMSO system , starch dispersions with concentrations lower than 10% (w/v) and higher than 20% (w/v) in formic acid were incapable of forming fibers, even after aging [7, 14, 15]. This result occurs even though amylose was found to form a transparent solution in concentrations up to 25% (w/v) in formic acid [13].

While many studies exhibit the importance of apparent amylose content, little work has been done in regards to the effect of molecular structure on the ability of starches to be made into nanofibers through electrospinning. It was shown that the molecular structure of starch influences the average achieved fiber diameter based on the amylose

and whole starch molecular size along with the amylose chains' degree of polymerization [16]. This study investigated 11 different starches with different amylose contents. For each starch, the researchers prepared samples of nanofibers via electrospinning using the same parameters. A solution of 17% w/v in FA was electrospun with a voltage of 15-20 kV, flow rate of 0.2 mL/h, and a distance from the needle to the collector of 15 cm. These conditions yielded fibers ranging in diameter from 126.6 – 843.5 nm, and these were collected on a grounded drum rotating at 5 rpm. From there, the shear viscosity, molecular structure, and electrospinnability were measured. Size exclusion chromatography was used to determine the hydrodynamic radius of amylopectin and amylose for each sample, as well as their chain length distribution. Amylopectin's molecular structure played a significant role in fiber quality based on droplet number and shear viscosity. It was found that fewer short amylopectin chains and more long amylopectin chains led to a lower droplet number. On average, a lower degree of branching and a higher degree of polymerization for amylopectin chains lead to smaller droplet numbers. Furthermore, the shear viscosity was correlated to the amylopectin-related molecular structure. Shorter amylopectin chains and a higher degree of branching led to greater shear viscosity. On the other hand, higher values of amylopectin's average degree of polymerization and ratio of long amylopectin chains led to lower values of shear viscosity. The molecular structure of amylose also contributes to the quality of the fibers attained. Lower hydrodynamic radius of amylose and whole starch and a lower degree of polymerization lead to slower fiber diameter. These same parameters affected shear viscosity and surface tension. Overall, the molecular structures of amylopectin and amylose were shown to play a significant role in the electrospinning of starch fibers.

Electrospinning Setup and Parameters for Favorable Fiber Characteristics

The third major challenge in electrospinning pure starch fibers is optimizing their physical characteristics through refining the setup and parameters of the electrospinning process. Many previously achieved pure starch fibers are not optimal. In order to realize the full potential of starch fiber mats, their mechanical strength must be improved. A study was done using a custom-made wet-electrospinning setup with a drum collector to increase the tensile strength of the starch fiber. Three operational parameters were tested: rotational speed, drum location, and coagulation bath composition [17]. The tensile strength was related to fiber alignment and was influenced by the interaction of location and rotational speed with ethanol concentration. This is similar to the relationship between fiber orientation and tensile strength found in other dry-electrospinning studies [18, 19]. In addition, this study showed that higher rotational speed and lower ethanol concentration led to improved fiber alignment. This inexpensive laboratory device did not significantly reduce fiber diameter; therefore, the improvement in tensile strength can be attributed to fiber alignment. The conditions for best alignment and weight normalized tensile strength include drum rotation at a speed of 500 rpm placed below an 80% ethanol coagulation bath. The tensile property of starch fiber mats has also been found to be affected by storage conditions. Fiber mats equilibrated at relative humidity >75% over 28 days exhibited significantly increased weight normalized tensile strength compared to unconditioned as-spun mats and mats stored at lower relative humidity [20]. The fiber size and crystallinity did not undergo any significant change based on morphological observation and X-ray diffraction analysis, and the attachment of intersected fiber segments, or conglutination, from the plasticizing effect of water is thought to be the cause of mechanical improvement. Starch fiber mats with improved mechanical strength are not yet strong enough for commercial applications, but future development is promising.

Additional research was conducted to investigate the methods used to increase strength in starch fibers fabricated through electrospinning. This research focused on increasing the tensile strength, decreasing sensitivity to water, and maintaining a non-cytotoxic fiber through the incorporation of glutaraldehyde in the vapor phase [21]. Glutaraldehyde vapor was administered post-

treatment as a way to form crosslinking between the starch nanofibers. The researchers tested the effects of exposing starch nanofiber membranes to crosslinking in glutaraldehyde for different amounts of time. The conditions for glutaraldehyde testing include 25% aqueous glutaraldehyde at 37 °C. Starch fiber mats were affected by the glutaraldehyde vapor crosslinking. The membranes developed a yellow color as opposed to white. Furthermore, the membranes shrunk as a whole, while the individual nanofibers increased in size. Fourier transform infrared spectroscopy was used to examine the samples, and this imaging indicated crosslinking between the fibers. Allowing glutaraldehyde crosslinking for 24 hours showed positive results in comparison to no crosslinking or crosslinking for only 12 hours. The 24-hour crosslinking condition yielded a product capable of resisting dissolution in water. The crosslinked starch nanofiber mats were shown to possess close to 10 times the tensile strength of those that were not crosslinked, and they were non-cytotoxic. The results of this study indicate that the alignment of fibers, specifically the intersection or crosslinking of fibers, is significant in inducing mechanical improvements in areas such as tensile strength.

In addition to exploring parameters which increase mechanical strength in fibers made from standard pure starch starting materials, it is important to understand optimal parameters for the fabrication of fibers from a variety of pure starches. For this reason, other pure natural starches were investigated for their possible unique electrospinning characteristics using related methods. One study sought to find the optimal conditions for electrospinning starch nanofibers from native yam starch [22]. The use of a 20 kV applied voltage, a spinning distance of 20.5 cm, and a flow rate of 1 mL/h was shown to be the optimal conditions for producing nanofibers from yam starch. These fibers averaged a diameter of 134.1 nm with a 16.71 nm margin of error, and the apparent amylose content was shown to be 33.7%. Another natural starch, tapioca starch, was successfully electrospun with a unique collection and dehydration technique that involved an ethanol collector cooled to -20 °C [23]. This study tested a range of solutions of natural tapioca starch and deionized water from 3-5% (w/v). Each sample was fabricated using an applied charge of 20 kV, a flow rate of 10 mL/h, a distance of 15 cm from needle to collector, and a 0.9 mm tip diameter. This study placed a metal target for fiber deposition in a -20 °C ethanol bath to more effectively dehydrate the fibers, and fiber mats were formed with the exception of the condition with a 3% tapioca starch solution. These fibers ranged in diameter from 1.3-14.5 μm. Out of the samples collected, Fourier transform infrared spectroscopy spectra and X-ray diffraction studies pointed to the heightened crystallinity in the 4.5% (w/v) sample. Furthermore, this same parameter was shown to have the greatest surface area and water swelling value. Both of these studies exhibited the possibility of successful fabrication of pure starch fibers from diverse, natural sources and indicated methods for electrospinning such fibers based on the unique starch characteristics of both yam and tapioca starch.

Conclusion

Starch shows incredible potential as a starting material for electrospinning fibers due to its availability, but three main challenges stand in the way of realizing this potential. First, starch must be broken down from its natural, granule form to an acceptable form for electrospinning Both the DMSO and the formic acid methods have shown potential in accomplishing this task. Next, the nature and effects of different starch compositions, especially the amylose:amylopectin ratio must be investigated. Research has shown that, in comparison to amylopectin, higher amylose content, in general, has positive effects on pure starch fiber formation, but there is potential for amylopectin to positively contribute to pure starch fiber electrospinning through hydrolysis and/or aging. Additionally, the molecular structure of both amylose and amylopectin must be considered when fabricating pure starch fibers. Finally, the optimization of the electrospinning setup and other parameters for fibers with desirable characteristics, such as increased mechanical strength, must be achieved. In this case, methods which focus on fiber alignment have shown positive results for

mechanical strength. While progress in addressing the challenges facing the electrospinning of pure starch nanofibers is promising, improvement is still necessary to maximize use of pure starch as a starting material in electrospinning nanofibers.

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Studying the Effects of E-Cigarette Chemicals on the Body Using the Model Organism *Drosophila melanogaster*

Megan Hawks[1], Dr. Stanislava Chtarbanova[1]

Faculty Advisor: Dr. Stanislava Chtarbanova[1]
[1]*Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487*

E-cigarettes have become seemingly popular among younger adults over the last decade; however, little research is reported on the use of these devices. This has brought to question the health hazards and side effects associated with vaping/e-cigarettes. For instance, chemicals used in e-cigarettes when aerosolized can be converted into carbonyl compounds, ketones, aldehydes and other compounds exerting detrimental effects on human health. This review will discuss how the effects of the chemicals found in e-cigarettes disrupt organ systems in the body such as the central nervous system (CNS), the cardiovascular system, and innate immune system in model organisms used in research with a primary focus on Drosophila melanogaster. This review will also analyze currently used treatments and discuss the need for additional research not only on the e-cigarettes long-term effects but also treatments for the negative effects of these devices.

Introduction

The second leading cause of death in the United States is stroke. 87% of strokes are ischemic, caused by an interruption of blood supply to the brain resulting in brain infarction and cell death. The detrimental effects of strokes are irreversible, oftentimes leaving patients permanently disabled or even dead [12]. Currently, 18% of all deaths are directly attributed to smoking, while 30% of deaths are cancer related. Studies have shown that 90% of adult smokers admit to smoking before the age of eighteen [23]. These overwhelming statistics raise concerns for the detrimental long term effects cigarette smoking can have on the human body, specifically in adolescents. E-cigarettes produce an aerosol by heating up a liquid which contains nicotine-an additive drug which is used in other tobacco products such as cigarettes and cigars. E-cigarettes were designed to benefit adults who already smoked to veer away from tobacco products, but still not enough studies are done to truly determine if e-cigarettes are effective in getting smokers to quit smoking. According to the Centers for Disease Control and Prevention (CDC) e-cigarettes are not beneficial at all to youth, young adults, pregnant women, or people who did not smoke previously [3]. In recent years (2014-2018) the percentage of adolescents (18-24) smoking e-cigarettes has increased by 46.2%. An increase in highschoolers using e-cigarettes jumped from 1.5% to 20.8%. Similar results have been described globally in places such as Great Britain, Taiwan, and Hong Kong [20]. The two main chemicals found in e-cigarettes are formaldehyde and ethylene glycol, which can cause chronic brain inflammation which could allow these neurotoxic chemicals to cross over the blood brain barrier (BBB) [22]. In addition to inflammation, e-cigarettes are known to cause oxidative stress and mitochondrial dysfunction [20]. Long term side effects of e-cigarette usage significantly impairs the body's vascular system, increasing the risk of cardiovascular disease.

In adolescents, the central nervous system is under constant development and is dependent on intense experiences in order to properly develop the prefrontal cortex [1]. Studies have shown that the use of nicotine results in diminishing cognitive function, such as reduced attention span and enhanced impulsivity, an increase in anxiety and fear, as well as depression later in life. Statistically, adolescents are more likely to do drugs, specifically cocaine and alcohol, engage in sexual behaviors and develop psychiatric behaviors such as obsessive-compulsive disorder

(OCD), anxiety, depression, and attention-deficit/hyperactivity disorder (ADHD) [23]. ADHD is a neurodevelopmental disorder in which patients experience symptoms such as having trouble paying attention, controlling impulsive behaviors, or being overly active [4].

E-cigarette aerosols that are generated through the heating up of liquid chemicals with nicotine in them are thought to have adverse effects on the nervous system, cardiovascular system, respiratory system, and gastrointestinal tract. Aerosols have shown to increase proinflammatory cytokines causing gene immunomodulation in a variety of human organ systems [16].

Some people who smoke report feeling a "buzz" or get a head rush while using e-cigarettes. The constant inhalation of chemicals causes a cutoff of oxygen and blood supply to the brain. The lack of blood and oxygen in the brain causes chronic inflammation and ischemia in the brain. Neuroinflammation is a process whereby the innate immune system is activated following inflammatory challenges. Chronic neuroinflammation activates additional microglia, promotes their proliferation, and results in further release of inflammatory factors such as elevated levels of several types of cytokines [8].

Injured cells in the ischemic brain, can release danger-associated molecular patterns (DAMPs) which also activate the innate immune system and inflammatory response. Inflammatory mediators released from brain cells could then spread to the blood vessels and amplify the expression of inflammatory mediators, which then drives the influx of leukocytes into the ischemic brain aggravating the inflammatory response [12].

Prior acute usage of e-cigarettes has been shown to cause inflammatory and cardiopulmonary physiological changes, while chronic usage has shown increased pulmonary effects as well as neurotransmitter alterations in reward pathways [16]. Chronic inflammation and elevated levels of cytokines are associated with neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) [8]. The increased inflammatory response and innate immune response dramatically expand brain damage, making e-cigarette smokers more susceptible to ischemic strokes, chronic inflammation, brain hemorrhaging, neurodegenerative diseases, and much more susceptible to infections due to the damaged BBB [12].

In human adolescents, nicotine usage has been linked to quinpirole-induced penile erection, locomotion and acquisition of cocaine self-administration which occurred via dopamine receptor (D3) enhancement through corticotropin releasing factor (CRF-1) activation. Nicotine exposure in adolescents leads to enhanced anxiety, increase in depression, and diminished cognitive functions as adults, reducing attention span and enhanced impulsivity. Adolescents who smoke are consistently more likely to do drugs, engage in high-risk sexual behaviors and develop psychiatric behaviors [23].

On a more physiological level, use of nicotine in adolescents has shown that even a low dose of intravenous treatment has immediate effects on serotonergic function, with elevated Serotonin Transporter (SERT) binding. Nicotine exposure has been found to lead to dendritic remodeling in prelimbic cortex and nucleus accumbens shell. E-cigarette smoke causes mitochondrial dysfunction, inflammation, oxidative stress, deregulation of calcium and neurotransmitter homeostasis [20]. This is a rising threat to current generations and generations to come as smoking has been discovered to cause multigenerational altercations in the epigenome [23]. Preclinical model organisms used in biomedical research which share similarities with humans at the genome level could help to characterize the effects of e-cigarettes at the molecular level and inform novel treatments to improve health outcomes associated with smoking and e-cigarette consumption.

Existing Research Models to Investigate the Effects of E-cigarette Chemicals

To date, both vertebrate and invertebrate models such as rats or the common fruit fly *Drosophila melanogaster* respectively, are used in researching the effects of exposure to e-cigarette chemicals. This review will focus on

the use of the invertebrate *Drosophila* model. *Drosophila melanogaster* is a well-studied biological model used to study biomedical science at a low cost, with rapid generation time. 60% of *Drosophila* genome corresponds to that of humans, making them an excellent model for studying human diseases [5].

Additionally, *Drosophila* allows us to explore molecular underpinning of immune reactivity. *Drosophila melanogaster* respond to septic injury by activating a complex innate immune response (flies lack classic adaptive immunity) that is similar to the mammalian innate immune response [11, 22]. The main systemic immune cells used in *Drosophila melanogaster* are hemocytes, which are macrophage-like cells or migratory phagocytes. Through the process of phagocytosis these cells can remove pathogens from the circulation system, or through the secretion of antimicrobial peptides (AMPs) which specifically target pathogens. AMPs are also produced and secreted in the hemolymph by fat body cells, which is an equivalent to vertebrate liver function. The expression of AMPs in *Drosophila* is triggered by the activation of the Toll pathway and the immune deficiency pathway (Imd) which are centered on the activity of the nuclear factor kB (NF-kB). Macrophages within the brain can phagocytose synaptic material, reducing locomotor abilities and longevity [22]. A recent study done on infected *Drosophila melanogaster's* brain both ex vivo and in vitro showed that several mammalian neurotropic pathogens can pass across the BBB. The study showed that hemolymph-borne pathogens invade the nervous system in response to the antibacterial response. In *Drosophila* NF-kB pathway activation can result in expression of platelet-derived growth factor (PDGF)– and vascular endothelial growth factor (VEGF). Two ligands of the fly's PDGF/VEGF receptor homolog, Pvf2 and Pvf3, are involved in the massive influx of macrophages and negative regulation of the Imd activity. Pvf2 expression in either glial cells or neurons is sufficient to allow migration of macrophages into the central nervous system [22].

The BBB guarantees metabolic and ion regulation in the brain preventing pathogens from entering the nervous system. Hemolymph-resident macrophages are able to migrate across the BBB via immunity induction of glial cells. While the Imd pathway can trigger expression of Pvf2 to guide migration across the BBB, macrophages' ability to increase immune response and cross the BBB reduces longevity and causes brain damage [22].

E-cigarette devices are metal or plastic tubes that contain a cartridge filled with a liquid that is vaporized by a battery-powered heating element. Previous studies done on the effects of e-cigarettes in *Drosophila* have shown that two main non-nicotine components, vegetable glycerin (VG) and propylene glycol (PG), have detrimental effects on the flies [5]. When PG and VG are heated, they can form a variety of carbonyl compounds such as formaldehyde, acrolein, and acetaldehyde. In one experiment using a nebulizer for 18 seconds over the duration of two days on *Drosophila*, the flies' experimental groups had a significant decrease in offspring compared to the control. The size of flies in the F1 generation was significantly smaller in ⅔ of the experimental groups in the parent generation. All F1 experimental groups were statistically more likely to develop at least one phenotypic change such as change in body color (referred by the authors as 'phenotypic mutation') when exposed to PG and VG. Almost immediately after exposure changes in reflex and activity patterns were observed [5].

In another experiment from the same *Drosophila* study, a volumetric pipet and bulb were used to administer 10 puffs of e-cigarette vapor to the flies, the results showed that 93% of the flies developed at least one phenotypic mutation and the number of offspring decreased by 80% [5]. An additional study that observed that effects of e-cigarettes on maternal offspring of *Drosophila melanogaster* showed developmental delays between the larvae and pupal stage resulting in lower weight and size of offspring [6].

In their study, Dosi et al. [5] discuss that their results aligned with work done in mice. Mice that were exposed to nebulized propylene glycol and vegetable glycerin non-nicotine aerosols for 13 weeks, 5 days a week, for 6 hours a day showed altered lipid balance in the lungs in ways that decreased the ability of the lung macrophages to fight infections. This effect

did not depend on the nicotine, but was related to the propylene glycol and vegetable glycerin that are the primary carriers in e-cigarettes [14]. However, all the mice showed an increase in weight over a 90-day period. Males had a slightly lower body weight in groups with VG, PG, and nicotine whereas the females had higher body weight than those that were not exposed to nicotine [5]. The study mainly focused on the toxic effects of the aerosol on the liver, respiratory system, and blood chemistry of the mice in a single generation. Additionally, changes due to stress in behavior and weight were also observed in the mice specifically in the females.

In another model where they tested nicotine effects on the adolescent brain in rats, high doses of nicotine in adolescents resulted in altered indices of serotonin receptor function, this was also noted as a long-term effect. Adolescent rats exposed to nicotine were quinpirole induced; causing an increase in locomotion and sniffing and induced compulsive behaviors that were symptomatic of obsessive compulsive disorder (OCD) seen in humans [23].

E-cigarette Chemicals used in *Drosophila* Studies

Formaldehyde

It is important to understand how inflammation and an increased immune response aids in the migration of macrophages and pathogens across the BBB when discussing the effects of e-cigarette or cigarette smoking on the brain. Two of the main chemicals found in e-cigarettes are formaldehyde and ethylene glycol. E-cigarettes produce carbonyl compounds such as formaldehyde, acetaldehyde, acrolein, and glyoxal. Glyoxal has shown mutagenicity [18].

A study done in *Drosophila* tested the effects of formaldehyde on development of larvae [9]. The results showed it took larvae significantly longer to climb the culture tube wall than the control group. The number of female *Drosophila* in the F1 generation was significantly lower than the control group, but the male *Drosophila* showed no significant change in F1 generation. Of the *Drosophila* that were exposed to formaldehyde, some had turned hard and black which indicated death; thus, formaldehyde has a toxic effect on development and growth. 20 flies were measured by their body weight at 3 days of age from each F1 generation, and while there were no significant changes in the males' body weight exposed to formaldehyde compared to the control, results did show that females exposed to formaldehyde were smaller and weighed less. Results also showed that *Drosophila* exposed to formaldehyde have a shorter life span than those in the control. Climbing ability of the females exposed to formaldehyde was significantly lower while in males it was slightly reduced. The results indicated that exposure to 0.15% formaldehyde for 3 minutes, 5 minutes, and 8 minutes leads to reduced learning and memory abilities in *Drosophila*. This same study later assessed the quantification of Brain Derived Neurotrophic Factor (BDNF) among each of the groups [9]. BDNF is a highly regulated molecule involved in changes in memory and learning. Changes in BDNF expression is linked to psychological disorders and pathological aging specifically in areas such as the hippocampus and parahippocampal area [15]. The levels of BDNF were sampled from brain tissue of the *Drosophila*. In the group that was exposed to formaldehyde expression was significantly lower than the control. However, a group that was exposed to formaldehyde and treated with docosahexaenoic acid (DHA), a fatty acid necessarily for development, was significantly higher than the control group [9]. These studies suggest that formaldehyde exposure results in negative effects on growth and development, reproductive ability, survival time, motor ability, and learning and memory function, which are consistent with the evidence in other animal models. Mechanisms related to FA-induced toxicity is not well known; however, it does appear that DNA and the chromosome may have been damaged consequently to the enzymes, proteins, and hormones have been altered, and thus, the reproductive, locomotive, and brain organs of FA-induced *Drosophila* have been dysfunctional [9].

In another study where *Drosophila* were exposed to formaldehyde and toluene in an inhalation chamber, results showed that 37 and 130 genes were expressed differently after exposure to these two chemicals, but more genes

were affected by formaldehyde than toluene. The subsequent gene ontology (GO) analysis performed on the differently expressed genes (DEGs) revealed that the immune response was one of the most significantly overrepresented GO terms for both toluene and formaldehyde exposure [7]. This suggest both chemical trigger the immune response even in the absence of pathogens. In humans, formaldehyde exposure has shown to decreased white blood cell counts [10].

The amount of carbonyl compounds among e-cigarettes not only varies between manufacturers but also between different samples of the same products meaning the concentrations of these toxic chemicals is poorly regulated and measured. A very high level of formaldehyde was found in e-cigarettes aerosols compared to toxicants found in combustible tobacco cigarettes [18]. Formaldehyde is thought to play a critical role in mediating neurodegenerative diseases such as Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis (ALS) and brain cancer. High concentrations of formaldehyde exposure have shown to have a 78% increase in developing ALS, a nervous system disease that breaks down nerve cells and reduces functionality of muscles the nerve cells supply. Formaldehyde exposure also causes a 71% increase in developing brain cancer. Formaldehyde induces misfolding of neuronal tau in vitro [19]. Chronic formaldehyde exposure leads to memory impairment. Abnormal elevations of endogenous formaldehyde have also been found in the brain pathologies of patients with Alzheimer's. Formaldehyde can cause age-related memory loss, which can act as a factor when triggered Tau hyperphosphorylation via GSK-3β catalysis including polymerization of Tau. Under formaldehyde stress Tau becomes hyperphosphorylated in not only the cytoplasm but the nucleus of neuroblastoma cells as well [13]. Formaldehyde is classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer [18]. It is projected that by 2026 formaldehyde is expected to reach 36.6 million tons making this a major public health concern [19].

As for the devastating effects of formaldehyde on the brain there is no known antidote for treating patients who have been exposed to formaldehyde (FA). Because DHA is known to have beneficial effects on health including enhancements in memory and learning, in a study using flies it was found to increase the reproductive processes in *Drosophila*, increasing the number of eggs, larvae, and adults [9]. DHA was also found to have a mild protective effect against FA-induced impairments in memory and learning. The results showed that the flies exposed to 0.15% FA significantly declined in the fecundity compared to the control group. DHA was then added in different concentration levels to the FA-exposed flies and not only did the number of eggs and the number of flies increase but it also influenced the rate of growth and development of offspring. The group that was given FA and then later treated with DHA was significantly faster in climbing back up the vial than the group that was treated with just FA. We can assume now that the FA inhibits locomotor abilities and delays development in *Drosophila* but DHA can counteract this phenomenon. Treatment of DHA also decreased the number of pupae that died. In the future this study should be carried out in a larger model such as rats or even monkeys to see if DHA has such a large effect on a more closely related model to humans [9].

Ethylene Glycol

Ethylene glycol is a clear odorless liquid commonly used as antifreeze in cooling and heating systems, hydraulic brake fluid and is also used as an industrial solvent. Researchers have identified ethylene glycol in samples of e-cigarette products despite the fact it was nowhere to be found on any of the labels or ingredients. Some e-cigarette manufacturers produce products with an ethylene glycol concentration as high as 76% [18]. When the effects of ethylene glycol were studied in mice, there were two groups, one which included nicotine and one without nicotine, after four weeks the neurotoxic chemical, ethylene glycol caused cognitive neurodegeneration with even more pronounced effects in the group without nicotine. Cytotoxicity was not correlated with nicotine levels but rather with the number and concentration of flavor chemicals. E-cigarette

and aerosol liquid exposures altered mitochondrial dynamics, membrane potential and caused hypoperfusion and swelling. E-cigarette exposure induced increased production of mitochondrial superoxide [20].

Ethylene glycol ingestion has shown damages in the brainstem and midbrain. Damages to these parts of the brain have had severe effects such as coma, cerebral edema, cranial nerve dysfunction, centrally mediating vomiting and hypertension. Ingestion of ethylene glycol has shown to lower density of the basal ganglia, thalassemia, midbrain and upper pons area of the brain. Ethylene glycol ingestion has been known to cause bilateral putamen necrosis, ultimately leading to death of most cells [17]. Symptoms of ethylene glycol ingestion can begin as soon as 30 minutes to 12 hours, causing seizures of status epilepticus. Pathology reports of patients who had an over exposure to ethylene glycol showed cerebral swelling, brain hemorrhaging, aseptic meningitis, encephalitis and crystal formation in the central nervous system. Ethylene glycol blocks the conversion of glycolic acid to glyoxylic which induces metabolic acidosis by a high ratio of NADH:NAD. Necrosis was found in areas of the optic nerve and in some areas of cerebral white matter, a similar physiological effect of those with carbon monoxide poisoning. Plasma levels and gastric juice were excised from patients with over exposure to ethylene glycol and analyzed. Results showed an increase in visual disorders, high anion gap metabolic acidosis, and elevated levels of creatine kinase [21].

Flavorings

In 2014 it was reported that there were 7,000 unique e-liquid flavors available for e-cigarette products on the market. Almost no studies have been done on the effects of exposure to flavorings. In most cases flavorings aren't even listed in the ingredients or labels of the e-cigarette products, most will say “natural or artificial flavoring”. The Flavor & Extracts Manufacturers Association says that many flavors are GRAS in food products, at their levels of intended use. But these ingredients have not been safety tested for alternative exposure routes other than ingestion, such as inhalation or aerosolization. One example is that saccharides are a known sweet e-liquid, but when they are degraded and when heated they produce furans and aldehydes. Formation of aldehydes have been found in several e-cigarette products and are known to cause respiratory irritation [16].

Another common flavor amongst e-cigarette users was menthol. Menthol flavored products contained chemicals such as pulegone and eucalyptol. Menthol properties include cooling and local anesthesia, effects drug absorption and metabolism, bronchodilation, respiratory changes, and electrophysiology. Similar concentrations of menthol were found in traditional combustible tobacco cigarettes [16].

Diacetyl, acetylpropionyl (also known as 2,3-pentanedione), and acetoin are chemicals used by food manufactures to add creamy flavors like butter, caramel, butterscotch, piña colada, and strawberry to food products. At least one of these three chemicals is found in over 90% of e-cigarette products. These chemicals have been associated with adverse respiratory health outcomes when aerosolized. Investigations have found an increase in cases of chronic cough and bronchitis, asthma, and bronchiolitis obliterans, a severe lung condition that can result in permanent pulmonary scarring and obstruction [16].

Another popular flavor is cinnamaldehyde-containing e-liquids which give a cinnamon, sweet, caramel, and fruit flavor. This study revealed using gas-chromatography mass spectrometry method, that more than half of the refill liquids possessed concentrations of cinnamaldehyde at levels that were deemed cytotoxic. Cinnamaldehyde depolymerizes microtubules of human pulmonary fibroblasts. Cinnamaldehyde is also found to decrease cell growth, attachment, and spreading; altered cell morphology and motility; increased DNA strand breaks; and increased cell death. Even at low concentrations cinnamaldehyde in e-cigarette products is proven to be cytotoxic and genotoxic and adversely affects cell processes and survival.

Benzaldehyde, which produces a fruity flavor, was found in 75% of e-cigarette flavors. Benzaldehyde is linked to irritation of the eyes and mucous membranes [18]. Prior acute e-cigarette usage demonstrated inflammatory and

cardiopulmonary physiological changes whereas chronic e-cigarette uses showed increased pulmonary effects, and neurotransmitter alterations. A study done on mice, tested the effects of inhalation of pod-based aerosols from a popular e-cigarette brand, JUUL, 3 times daily over a duration of three months on inflammatory markers in the brain, lungs, colon, and heart. Results showed that the JUUL aerosol exposure induced an upregulation of chemokines and cytokine gene expression such as HMGB1 and RAGE [16]. HMGB1 is extensively bound to DNA and is involved in transcriptional regulation, DNA replication and repair, telomere maintenance and nucleosome assembly. Lack of maintenance and regulation on telomeres will ultimately lead to incidences of disease and poor survival. Studies have shown changes in the myocardium of e-cigarette smokers. E-cigarette users experienced fibrotic changes in their cardiac tissue. Fibrosis is associated with cell injury or inflammation. An increase in pro-inflammatory cytokines and fibrosis-associated proteins have been linked to cardiovascular disease. In studies to assess which genes were associated with myocardial infarction and the development of heart failure, the results suggested that effects may be flavor specific and nicotine specific [16].

E-cigarette aerosols deposit into the retro- and oro-pharynx which leads to entry of these inhaled chemicals into the gastrointestinal tract (GI). Studies suggest that aerosols modulate inflammation in the colon, with induction of key inflammatory cytokines with acute exposure. Inhalation of these chemicals has proven to cause inflammation and immunomodulation which is seen through alterations of phenotypes of cells throughout the lungs. Gene expression changes are most likely caused by the flavoring chemicals. Overall, studies show that e-cigarette aerosols induce immunomodulation across several organ systems [16]. Future studies in genetically tractable organisms such as *Drosophila* could be useful in providing additional insights into the molecular mechanisms associated with the negative effects of e-cigarettes flavoring on health.

A study was conducted through a survey of e-cigarette smokers and the results showed that more than half of e-cigarette users reported that limiting the range of flavors available for e-cigarettes would only increase their cravings for traditional combustible tobacco cigarettes and would decrease the likelihood of quitting or reducing smoking habits [18].

Future Studies

E-cigarette usage results in ischemic conditions in the brain which could lead to ischemic strokes. In recent years, researchers have tried treating ischemic strokes through traditional treatments which are based on antithrombotic therapy and neuroprotective therapy which are greatly limited due to their poor safety and treatment efficacy [12]. The standard in traditional medicine for treating ischemic stroke is thrombolysis with tissue plasminogen activator to recognize recanalization to the brain. Except the time window for thrombolysis is extremely narrow being less than or at 4.5 hours due to the risk of hemorrhagic formation caused by low targeting ability to thrombus. Thus only a few patients are able to benefit from this treatment. More importantly, though, restoring blood flow to an ischemic brain would cause secondary reperfusion injury. Reperfusion would exacerbate the production of reactive oxygen species (ROS) and the amplification of inflammation and immune response, which would lead to neural death, impaired the integrity of the BBB and eventually lead to brain edema. Ultimately, leaving the brain much more damaged, if not dead, and more susceptible to bacteria entering the brain. Most neuroprotective approaches have undeniable defects such as low solubility, short half-life and poor BBB permeability in vivo. It has been studied that e-cigarette and aerosol liquid exposures altered mitochondrial dynamics, membrane potential and caused hypoperfusion and swelling [1,4]. So traditional treatments for ischemic strokes would not be a viable treatment for E-cigarette users due to the aerosol liquids damaging physiological effects.

In contrast, nanomedicine has developed ways to increase poorly soluble drugs, improve stability while extending half-lives of these drugs in vivo. Another advantage to nanomedicine is that targeted modified

nanomedicines can aid drugs in crossing into the BBB and accumulate at the desired sites avoiding non-specific distribution. Targeted modification also allows for specific cells to absorb the nanomedicine, such as damaged neurons. Nanomedicines include liposomes, micelles, nanoparticles (NPs), emerging cell membrane coated NPs and exosomes, all of which have been applied in the preclinical studies for treating ischemic stroke. Nanomedicines with targeting abilities could be a future desired treatment offering a safer, lower dose of drugs and more effective alternative to traditional treatments of ischemic strokes [12].

In the treatment for ethylene glycol poisoning, people who suffered from detrimental effects, could be treated with ethanol as ethanol is a competitive substrate for alcohol dehydrogenase. Ethylene glycol is absorbed through the gastrointestinal tract, which is then oxidized by alcohol hydrogenase to glycolaldehyde in the liver, then converted to oxalic acid, glyoxylic acid, and glycolic acid. Ethylene glycol is exerted through these metabolites either directly or indirectly [21].

Ethanol has a higher binding affinity than ethylene glycol meaning ethanol is effective and inhibits the metabolism of ethylene glycol. Ethylene glycol is inexpensive and easy to obtain but it is extremely hard to dose and has sedative and behavioral effects. An alternative to this treatment is fomepizole (4-methylpyrazole) which is another known competitive inhibitor for alcohol dehydrogenase and prevents the formation of toxic acid metabolites. Fomepizole is easy to dose and administer, and side effects are very rare. However, it is expensive and not available in all hospitals. Hemodialysis is used to clear ethylene glycol and its metabolites [21].

As for the effects of e-cigarette aerosols on the digestive tract, not many studies have been done. In the future, hopefully, studies will focus on the disruptions associated with e-cigarette chemicals on the microbiome of the gastrointestinal system. Disruptions of interrupted intestinal microbiota are associated with inflammation, immune status, and gut boundary integrity, inflammatory bowel disease (IBS), colorectal cancer, diabetes, and atherosclerotic cardiovascular disease [2]. The gut microbiome is known to have effects on things such as diabetes and neurological disorders but more research is needed to be done to see if e-cigarette chemicals have an effect on disrupting the intestinal microbiota.

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Interview with Dr. McMichael

Benjamin McMichael, PhD
Associate Professor
The University of Alabama School of Law

JOSHUA Staff (JS): *Regarding your background, what eventually led you to become a professor at the University of Alabama Law School? Where are you from? Where did you go to college and what did you do prior to taking on this role?*

Dr. Benjamin McMichael (DBM): I am from Macon, Georgia. It is a town about 70 miles south of Atlanta. I am one of the few Georgians you will meet outside of Georgia who is not from Atlanta. I went to college at Wake Forest and majored in mathematical economics. Then, I went to Vanderbilt because they had a joint JD-PhD program in law and economics, and it was the best fit for me. That is where I got interested in healthcare law and healthcare economics. Lawyers tend to ask really interesting questions, while economists are very good at answering questions. The inverse is also normally true: lawyers are not very good at answering interesting questions, and economists are not very good at asking interesting questions in my personal opinion. It was nice to be able to do both sides. After graduate school and law school, I went to clerk on the 5th circuit, which is the federal appellate court with jurisdiction over Texas, Louisiana, and Mississippi. Then, I went back to Vanderbilt for a postdoc, and that is where I got interested in a lot of the stuff that I am still working on today: scope-of- practice laws, and organ transplantation. My advisor when I was at Vanderbilt for my post doc said, "Hey, I know this guy over at the medical center who is doing something with organ transplantation. Why don't you go over there and see what he is doing?" I was supposed to have a thirty minute meeting, and six hours later I had a new research job. I made my way to Alabama about a year and a half after that.

JS: *Was there anything at The University of Alabama that was interesting to you?*

DBM: I really like the south. I understand it, and I feel like it understands me. I really like being around the students. I have been around students at other colleges and universities, and I like the students at UA better than those other colleges and universities. I don't care how much people pay you, and I don't care how much research money you get; if you don't like the people you're around, you're going to be miserable. So I really like it here, because I am never miserable around the people here. They are always super nice and interesting to talk to, so that is my favorite part.

JS: *What does a typical day of research look like for you and the people you work with?*

DBM: It is mostly me sitting in front of my computer all day. I will have goals in mind that I never get done on any given day. I will figure out how to do one little part, Google something for 30 minutes and make it work. When something else goes wrong, I will Google it for 30 minutes and make that work. I will get mad at the technology, and it is never the technology's fault; it is always something I have done. It is just sitting in front of my computer trying to figure stuff out. Some of it is interesting: moving data back and forth. For all the transplant data, with that level of detail you get insights into things that no member of the public ever does. They make a lot of that data public, but they don't organize it in certain ways. You can really see what is going on on a month-to-month basis at these transplant centers. That is a lot of fun, but it is also a lot of code not working and having to fix it.

JS: *A lot of our readers are going to have a background in how data collection works in the natural sciences. Could you describe how you collect data in your research?*

DBM: I am not a data collector. I rely on data collected by others, so, I mostly analyze observational stuff. For example, the transplant stuff comes from an organization called The Scientific Registry of Transplant Recipients. It is an organization that is authorized under federal law. They collect data on all waitlist registrations, transplants, and follow-ups on anybody who got a transplant. They give me the big databases that I then organize and analyze. So it's not a situation where I go out and run experiments on humans. That is not to say that experiments aren't useful, but for big policy-type things, unless you're testing people's responses to a specific intervention, which I'm usually not, I'm usually interested in how systems respond, not individual people. How does the transplant system respond to this intervention? You can't run experiments on that scale without seriously violating ethics and laws. You don't have enough money to do it, so I don't gather a whole lot of my own data.

JS: *What does it take for an undergraduate to become involved in your line of work? What advice would you give to someone looking to pursue a PhD or JD in your field of work?*

DBM: So first, email me. I'm happy to talk. People think that it is mostly ability or intelligence, and those things certainly matter. You have to have the capability to do this type of work. But mostly, it's just, you have to develop an unhealthy obsession with some area that you just really want to spend your life delving into. I tell people this job is very much enabling me with my addictions. I have an unhealthy obsession with healthcare law and healthcare economics, and for some reason someone decided to pay me money to do that. It's really more about that. Do you really want to spend your life thinking about these topics, almost only these topics, talking about them with other people, teaching other people how they work. As long as you have the base level intelligence and ability, the primary qualification is you really want to do research.

JS: *We understand that some of your more recent work has involved state apology laws and medical malpractice; what led you to this field? Did you have any personal or professional experiences that drew you to this career path?*

DBM: Malpractice was kind of my first foray into healthcare law. That's where I kind of started when I was a younger JD/PhD student. Torts is one of the first classes you take, and that is where I first started being exposed to how the legal system interacts with the healthcare system. It was kind of a natural, this is where I'm starting things. It turns out there was, and continues to be, a lot of work to be done in this area. I got interested in apology laws because we have been talking about Tort reforms which are basically state statute interventions into the litigation system and the legal system, that makes it usually harder to hold doctors liable for their mistakes. There are good and bad reasons for why we would want to have those reforms, but a number of reforms that have been talked about for the past thirty years, they've been analyzed. Apology Laws were really kind of the new wave of Tort Reform. They took a different approach. Instead of just limiting damages or something like that, they rely on this existing experimental literature saying, "if you apologize, people are less likely to sue , and they are more likely to accept settlements, and they are more likely to accept lower settlements". So states found this out and said why don't we pass Apology Laws, and the law basically says, "If a doctor apologizes to you, you cannot admit that apology as evidence of liability in a trial." That all sounds well and good, and it was in an experimental setting, but in the real world, so to speak, they didn't work like people thought they would. Probably the best explanation we have, and we have pretty strong evidence, is that what's going on is doctors are apologizing, people can't use those apologies themselves in court. But if a doctor apologizes to you, that's a pretty strong signal that they screwed up, and so even though you can't use that apology you can go gather a bunch of other evidence. Because if you're injured after a surgery, it could be from

malpractice, it could just be bad luck, you happened to have an adverse reaction that was beyond anybody's control or you just had an adverse reaction that was within our control but technically wasn't malpractice. So an apology can signal it was the doctor's fault, so I can't use that apology but I can go gather a bunch of other evidence and call the doctor out. A signaling model, for your more economically-inclined readers.

JS: *You've written about the impact of the scope of practice laws on the nursing field regarding the opioid epidemic. Since this research has happened, has COVID-19 changed anything in this field, especially as it did exasperate the opioid crisis? Is there anything you couldn't include in your publications that you find important or more provocative?*

DBM: So with the COVID stuff, a lot of things changed because we basically upended our healthcare system, for good reasons. But one thing a lot of people don't know is that a lot of states enacted temporary waivers to restricted scope of practice laws, basically turning themselves into full practice authority states, which basically means nurse practitioners can do almost anything physicians can, legally. That had an impact on COVID. There are a few people working on this, but I am not personally working on this right now. People are arguing back and forth–did these waivers have an effect? Did they lessen the impact on COVID? One paper that I'm thinking of, I don't think it's been published yet, seems to have found compelling evidence that these waivers didn't impact COVID deaths, but they impacted all other deaths. Which basically suggests, NP's were stepping in and taking up some of the slack from all the doctors who had gone to work on COVID stuff. So if all your internal med docs, your pulmonologists, whoever, are over here concentrating on the COVID patients, people are still having heart attacks. They are still having diabetes. They still have these conditions that they had before the pandemic. Some of the evidence I have seen suggests – it's not proven yet because it hasn't gone through peer review – that when you had these waivers for NP's, these other deaths didn't happen to the same extent because they could step in and substitute for the doctors who gone over to do the COVID stuff. In terms of the opioid exasperation, that is an outstanding question. We are just now getting access to post pandemic data. 2021 data came online in late 2022. We expect to have more 2022 data in late 2023, and you really do need those couple of years post-pandemic to see how things have really changed. We don't quite have that yet. I'm looking forward to working on how the opioid epidemic evolved, particularly in different states with different laws, but I don't really have any clean answers on that quite yet.

JS: *As I understand it, your research and training are interdisciplinary and require both tools from the realms of economics and law. In your opinion, what does UA do successfully to foster interdisciplinary research and collaboration, and what could it do better?*

DBM: This place is so much better than any other institution I've ever been at. When I was at Wake Forest, we did interdisciplinary stuff and it was great, and my major was interdisciplinary. It's a great school and I'm not bad mouthing it at all, but we didn't have all the research that goes on here. It's a smaller school. Even at Vanderbilt, which prides itself on being interdisciplinary, I don't know how many students get a chance to be involved in that. Certainly, the faculty are very interdisciplinary at Vanderbilt. But here, you're the second person from the undergrad campus to contact me about law and economics. Actually, no, you're the fourth person. And just having the opportunity to work with undergrad students, they usually don't normally come over to law schools. That's kinda unusual. So that's been really great. The Randall Research Scholars Program – I realize they're not going to do this, and there's reasons not to do this – but if we could expand that to about 500 students so we could all have one, that would be great. So if you want to put that in your suggestions, let's have some more Randall Research folks. I worked with one for two years, and she's now at my program. She's a JD/PhD candidate at Vanderbilt, and she's just outstanding. So, just expanding that kind of stuff and inviting students to faculty meetings where we're talking

about, 'Okay, there's this really hard problem, and we need these six disciplines to be involved in this to really solve this problem,' I think would be great. So, I'm working with a group on campus that's doing a bunch of opioid research, it's based out of the Institute for Data Analytics. I've met with them a couple of times. I think they have nursing people involved, psychology people involved, business people involved, me involved, so UA does a lot of this really well, in my opinion. In terms of improving, it's just, take what you're doing and multiply by ten. I mean, we're a big public research university. We have the resources to actually do this kind of stuff, and so, in my opinion, the more we can push that the better. I'd love to see more JD/PhD students here. I understand we have one coming in next year. I'd love to see three or four coming through our ranks every year. That'd be wonderful.

JS: *And the last question I have for you is: What are the most troubling or shocking insights you've gained into the healthcare system throughout your career?*

DBM: So basically, our entire healthcare system is crap. You can quote me on that. It doesn't work like people think it does. It's not set up for patients. It's basically a mish-mash of a bunch of legacy systems that we kept a lot of the stuff that benefits very specific interests. For example, certificate of need laws. You basically have to get the state's permission to get a hospital bed in the state of Alabama despite the fact that we have fewer resources and less access to healthcare than most other states in the union. You still have to get the state's permission, and they have to say, "Yes, that works with our state's health care plan, you can add a nursing home bed or an OR bed." That makes absolutely no sense to me. That's just stupid. We also don't have enough doctors in Alabama, and yet we have restrictive scope-of-practice laws to prevent NPs and PAs from practicing. And I'm picking on Alabama: most states have certificate-of-need laws, most states restrict the practices of NPs and PAs, so it's certainly not unique to us, and I don't mean to suggest that. Focusing on a national system, the transplant system: we export a lot of organs from the south and midwest to the northeast and west coast because they have better access to healthcare and more money. So despite the fact that the south and midwest are fundamentally sicker and in more need of transplants, because we don't get people onto the waitlist, the system is rigged to export organs to richer, more urban areas. I could keep going and going and going. You know, the whole thing about price transparency: nobody would go into a gas station, say, "I want this candy bar," put it down, eat it, and three days later, they send you a bill for $100 for a candy bar. That's essentially how our healthcare system works. Nobody knows what anything costs, which creates all kinds of perverse incentives. It doesn't make any sense that that's the way we run things. And courts are complicit in this. I mean, courts will enforce contracts for healthcare services without a price term. That would never happen in any other industry. No court is going to enforce a contract for you to buy a million widgets and nobody tells you what the widget costs. So, I mean, if you want me to keep going for eighteen hours, I can, but basically everywhere you look in the healthcare system, something is going to shock and appall you because it's either not patient-focused or it's based on something we did in the 1880s and we haven't changed it since then. It's just crazy how disjointed our healthcare system is.

JS: *If we wanted to get a "introductory look" into a lot of the problems in a survey-type reading, where would you look?*

DBM: Marty Makary has a good book: *The Price We Pay.* David Hyman, he's at Georgetown, he has a couple of good books that I can't remember off the top of my head. I would probably start with Marty Makary's *The Price We Pay.* David Goldhill had a book a while ago, and it focused on the insurance system: *Catastrophic Care.* And no book is going to be able to give you an introduction to all the problems because basically the entire healthcare system is a problem, but those are a couple that I would start with.

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2024 Submission Guidelines

We accept articles from current undergraduate students at accredited universities. If you are a graduate student or recent alumnus of UA, we will consider your article if the majority of your work was conducted while you were an undergraduate at UA. Undergraduate students from other institutions may submit; however, priority will be given to those who conducted their research at UA.

1. Your name, email address, and phone number must be included.
2. Your submission must relate to science or health.
3. Your work must be sponsored by a faculty member.
4. The length of your submission must be between 2000 and 4500 words. We will accept longer submissions if the author can limit the submission to the required length for the publication, and any extra material is able to be published online.
5. Figures, charts, and graphs are allowed but not required. (Note: The color will be mostly black and white.)
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