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Pre-Pulse inhibition in mutated mice: Studying compromised microglial cells to discover new genetic connections to autism

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Pre-Pulse Inhibition in Mutated Mice:

Studying Compromised Microglial Cells to Discover New Genetic Connections to Autism

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PUBLIC PRESENTATION

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This work is dedicated to Janell, Scott, Connor,

McKenna, and Beanbag- my wonderful support system.

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Preface

When talking about people who have disabilities, it is respectful to use person-first language instead of identity-first language. This means we would say “a person who is paralyzed,” not “a paralyzed person.” However, when it comes to the autism community, most prefer identity-first language. This community highlights the fact that autism is an important part of their identity, and this is why they prefer “autistic person” over “person with autism.” The Autistic Self Advocacy Network (ASAN) expertly says that “we recognize the value and worth of that individual as an Autistic person- that being Autistic is not a condition absolutely irreconcilable with regarding people as inherently valuable and worth something” (Brown, 2011, para. 18). This being said, it is imperative to act as an ally to the autism community. In another article, the ASAN says, “An ally is a person with privilege on a particular axis who makes a conscious choice to work against oppression on that axis. *In this instance it’s a non-autistic person working with and for autistic rights*” (Sibley, 2012, para. 3). Because of these statements from the autism community and my role as an ally, identity-first language is used in this work.

Acknowledgements

It is a genuine pleasure to express my deepest and most sincere gratitude to my project advisor, laboratory instructor, and professor, Dr. Lincoln Gray. His support throughout this research and writing has been invaluable. It has been a privilege for me to work under Dr. Gray's guidance- Life's Good with an advisor such as him. I would also like to thank my readers, Dr. Emily Zane and Dr. John McNaught for their advice, expertise, and encouragement.

Thank you to the National Institutes of Health (NIH) for their generous financial support that has allowed this research to be conducted. Additionally, I would like to extend my thanks to my fellow undergraduate and graduate researchers, along with Dr. Mark Gabriele. Without your wonderful teamwork and dedication, this work would not have been possible.

Wholeheartedly, I thank James Madison University, the Honors College, and the Department of Communication Sciences and Disorders for equipping me with the knowledge and skills that aided in the completion of this project.

Lastly, my appreciation goes out to my family and friends, especially Caroline Willhite and Samantha Johnson. Thank you for inspiring me to be a better student, friend, and future speech-language pathologist every day.

Abstract

Microglial cells “play a pivotal role in refining neural networks during early critical periods” (Gabriele & Gray, 2020, p. 1). A disturbance in the functioning of these microglial cells contribute to specific characteristics of some neurodevelopmental disorders- including autism spectrum disorder. In this study, we used a mouse model to study disruptions in cell activity, as understanding the biological and genetic background of autism spectrum disorder could help us provide better treatment and therapy options to those diagnosed.

The mutated mice in this experiment have microglial cells with “compromised fractalkine signaling” (Gabriele & Gray, 2020, p. 4-5). We studied multimodal psychophysics in heterozygous, homozygous, and wild-type mice in order to further investigate the link between genetics and autism. Auditory, somatosensory, and multimodal stimuli were presented to the mice and we measured the startle response with an accelerometer. The goal was to measure if the mice expect the startle-eliciting-stimulus when given a pre-pulse “warning.” This is known as pre-pulse inhibition (PPI). We were looking to find a lack of behavioral response in the mutant mice- since that characteristic is descriptive of autism- and a significant response from the wild-type mice (Gabriele et al., 2021). This would confirm a connection to autism.

With this research, we are able to gain a deeper understanding of neurodevelopmental disorders. This data has the potential to influence the identification timeline, school accommodations, and future research into the early critical period and autism spectrum disorder.

1. Introduction

What is Autism?

Dr. Temple Grandin says, “I am different, not less,” Dr. Stephen Shore speaks, “if you’ve met one individual with autism, you’ve met one individual with autism,” and Kerry Magro states, “Autism can’t define me. I define autism” (Grandin, 2012, p. 14; Shore, 2018, para. 2; Magro, 2010, para. 1). But what do these statements truly mean? To answer this, it is important to have a clear definition of what autism is. According to the National Institutes of Health (NIH), autism spectrum disorder, or ASD, “affects how people interact with others, communicate, learn, and behave” (National Institute of Mental Health, 2022, para. 1). It is also important to note that autism spectrum disorder is known as neurological disorder as well as a developmental disorder. It is categorized this way because while it is neurological, symptoms tend to develop over the first two years of life (developmental) (National Institute of Mental Health, 2022).

Signs and Symptoms

While there is likely an endless number of signs and traits of autism spectrum disorder since it presents so differently throughout individuals, the following are the two domains of these characteristics. Signs and symptoms can fall into social communication and interaction difficulties, restricted or repetitive behaviors or interests, and other challenges. Social communication and interaction difficulties can include language delays, avoiding eye contact, lack of facial expressions, uses few or no gestures by 12 months, does not point or look where another person points by 18 months, and does not pretend in play (National Center on Birth Defects and Developmental Disabilities, 2021). According to Dr. So Hyun Kim and Dr. Catherine Lord, restricted or repetitive behaviors (RRB) can be “preoccupation with restricted patterns of interest, adherence to specific, nonfunctional routines, repetitive motor manners, and

preoccupation with parts of objects" (Kim & Lord, 2010, Lay Abstract section, para. 1). Examples of RRB include playing with toys the same way every time, lining up toys and getting upset when the order changes, repeating words or phrases over and over, following specific routines, and stimming by hand flapping, spinning in circles, and rocking their body back and forth (National Center on Birth Defects and Developmental Disabilities, 2021). While the following signs aren't used for diagnosis, some other characteristics may include "hyperactive, impulsive, and/or inattentive behavior; epilepsy or seizure disorder; unusual eating and sleeping habits; unusual mood or emotional reactions; anxiety, stress, or excessive worry; and lack of fear or more fear than expected" (National Center on Birth Defects and Developmental Disabilities, 2021, Other Characteristics section, para. 1). Some say that autistic individuals often "see the trees, but not the forest." This is known as Weak Central Coherence Theory (Kennedy Krieger Institute, 2019). The term was coined in 1989 by Dr. Uta Frith, a developmental psychologist. On the authority of the NIH, this theory describes how "[autistic people] report exceptionally quick and accurate perception of small visual details, but often have difficulty integrating the details into their overall view of the world" (Intramural Research Program, 2014, para. 1). While any number of these signs may be present in an autistic individual, it is imperative to remember that individual differences are at play, and each person is unique in their experiences and day to day life as an autistic individual.

What We Know

When it comes to autism spectrum disorder, we still have an immense amount to learn. However, the following includes some of what we do know. Dr. Penzes, a researcher at Northwestern Medicine, states, "what we learned is that not all this genetic risk is inherited. Many are called *de novo* mutations, which occur in the parent and are transmitted to the child.

We also learned that probably hundreds or maybe even thousands of genes contribute to autism, but they contribute in a very complicated way" (Feinberg School of Medicine Research Office, 2018, para. 2). He also goes on to mention that since autism is a spectrum disorder, the genetic mutations and environmental components are different in each individual. According to Johns Hopkins University, here is what we know about ASD: 1) Having older parents means a higher risk 2) Boys are about four times more likely to be diagnosed than girls 3) Environmental factors likely play a role and 4) Genes DO play a role (we just don't know which ones) (Johns Hopkins University, 2017; National Center on Birth Defects and Developmental Disabilities, 2021).

Research Objectives

Since there is still so much to learn, we have eagerly turned to research to help us gain a deeper understanding of the role biology and genetics play when it comes to autism spectrum disorder. In James Madison University's Computational, Speech, Sensory, Development & Diseases Lab, Dr. Lincoln Gray and Dr. Mark Gabriele are conducting research with hopes that it answers some questions about the biological background of autism. The three research objectives are as follows:

1. *Determine the spatiotemporal patterning of microglia and fractalkine/ complement expression relative to emerging inferior colliculus lateral cortex compartments.*
2. *Assess microglial and fractalkine/ complement involvement in sculpting distinct multisensory circuits.*
3. ***Determine if microglial cell dysfunction results in atypical response behaviors*** (Gabriele, 2020, Abstract Text section, para. 1).

As a student in the Communication Sciences and Disorders Department, I focused on researching for the third aim of the study, which aligns most with my major. The goal of this writing is to

report the research outcomes of the third aim and examine how they relate to autism (assuming they do, in fact, relate). Additionally, I seek to explain how this data can and will be used to deepen our understanding of autism spectrum disorder, which in turn can help professionals provide better therapy options, as well as a better understanding, in the field of speech-language pathology.

2. Biological Background

Microglial Cells

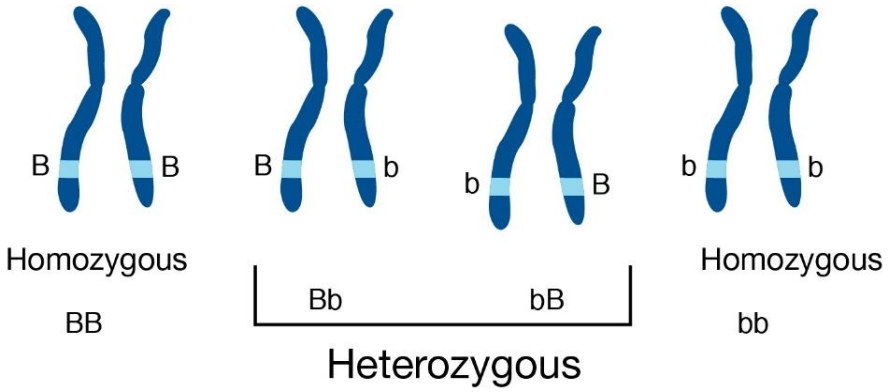
Microglial cells (MGC) "play a pivotal role in refining neural networks during early critical periods in a variety of systems" (Gabriele & Gray, 2020, p. 1). Disruptions in microglia signals are thought to contribute to autism spectrum disorder. This is because microglial cells are responsible for synaptic pruning in the early stages of development, which we call the "critical period" (Scott-Hewitt, 2020, para. 1). According to the Harvard Brain Science Initiative, "[microglial cells] play a critical role in synaptic pruning by targeting axons and synapses for elimination through engulfment of immature or less active inputs" (Scott-Hewitt, 2020, para. 1). A lack of this usual synaptic pruning means that many nonessential axons and synapses do not get eliminated. This is significant because it means that disruptions in microglial signaling may contribute to certain neurological or developmental disorders (Gabriele, 2020). Dr. Gabriele expertly states, "preliminary data from our lab suggests similar mechanisms influence the refinement of multisensory maps in the inferior colliculus, a midbrain structure known to mediate startle responses" (Gabriele et al., 2021, p. 1). Less synaptic pruning in the inferior colliculus (specifically in the lateral cortex) means more of a startle response to stimuli (both unimodal and multimodal) (Gabriele & Gray, 2020). If we are able to measure and analyze these startle responses and behaviors (between mutated and non-mutated systems), we can confirm the lack of synaptic pruning, which is a direct result of compromised fractalkine signaling and microglial cell dysfunction (Brett et al., 2021). This is crucial to our understanding of autism spectrum disorder because if there are atypical response behaviors in these systems, it shows that neurodevelopmental disorders (like autism) are influenced by microglial cell dysfunction (Gabriele & Gray, 2020).

Animal Models

This research was conducted through the use of animal models- more specifically, a mouse model. If we are able to demonstrate the disruption of microglial cell activity in mice, then we may be able to gain a deeper understanding of microglial cell activity in humans (especially in autistic individuals). Our research includes mice that are homozygous, heterozygous, and wild-type. Homozygous means they carry two identical alleles, while heterozygous means the alleles differ as indicated by Figure 1. "Wild-type" is the term used to

Figure 1

Heterozygous and Homozygous Genotypes



describe the mice that are not mutated (Gray, 2021, p. 6). The heterozygous and homozygous mice are the ones with "compromised fractalkine signaling (CX3CL1-CX3CR1)" (Brett et al., 2021, Abstract section, para. 1). They are the "mutant" mice (Gray, 2021, p. 6). Fractalkine signaling is responsible for the "functions of microglial cells in part by regulating their timely recruitment at sites of developing synapses" (Arnoux & Audinat, 2014, Abstract section, para. 1). Compromised fractalkine signaling means that the microglial cells at those synapses (that are imperative to maturation at early postnatal stages) can result in uncontrolled responses, which halts synaptic pruning (Gabriele & Gray, 2020). These outcomes in responses and synaptic pruning have been hypothesized to have connections to neuro-developmental disorders,

including autism spectrum disorder (Arnoux & Audinat, 2014). Replicating this effect in animal models, specifically a mouse model, has proven to be useful as we test their responses to stimuli in order to help us achieve a greater understanding of the consequences of compromised fractalkine signaling in the lateral cortex of the inferior colliculus (LCIC) (Gabriele & Gray, 2020).

3. Methods

Procedures

In the laboratory, there are specific procedures and guidelines that the volunteer researchers follow. First, we check the list of mice and pick one that has not been tested, or "run," yet. The codes for the mice look like the following: LA, UC, RT, etc. The first letter will be "L" for left ear tagged, "R" for right ear tagged, "B" for both ears tagged, or "U" for untagged. The second letter is the letter for which cage the mouse is in. This typically ranged from about "A" through "K." Once a mouse is identified as untested on the list, we put on our lab coat and gloves to safely handle the mouse. We go to the mouse room, find the correct cage, and select the correct mouse. It is important here to always double check for holes in the ears, as a tag may have fallen out. We gently let the mouse crawl into the tube, then secure the end piece. The tube with the mouse in it then goes into the sound booth on top of a shaker, as pictured in

Figure 2

Schematics of Multimodal Behavioral Setup

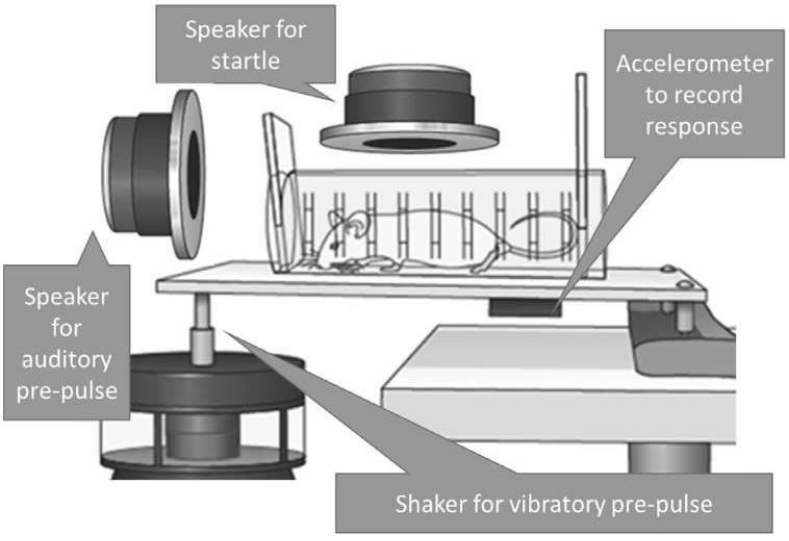


Figure 2. The sound booth is also equipped with speakers and an accelerometer. After all the equipment has been plugged in and turned on, it is time to enter the information in the computer

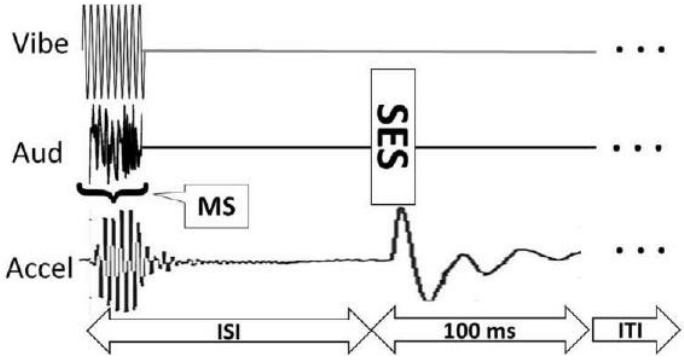
program. In the lab, we use MATLAB, which is "a programming platform designed specifically for engineers and scientists to analyze and design systems and products" (MathWorks, 2022, para. 1). In this program, we enter the mouse that we chose, our initials, and verify that all the information is correct. We click "save," then "run" in order to start the program. Importantly, we record the date on the mouse sheet to show that the mouse has been run. After a few minutes, we know the program is successfully working because there will be graphs of data that appear on the screen. This data shows the startle response to unimodal and multimodal systems.

Unimodal and Multimodal Methods

Unimodal means "one mode," while multimodal means "multiple modes." In our experiment, the unimodal method consists of either producing an auditory stimulus, or a somatosensory stimulus, but not both. By contrast, the multimodal method produces the auditory and somatosensory stimuli simultaneously (Gabriele & Gray, 2020). The auditory stimulus is best described as a short, but loud broadband sound. The frequency and intensity of which was specifically chosen from a mouse audiogram to ensure the mice would be able to hear the frequency (pitch) and intensity (loudness) of the stimuli (Brett et al., 2021). The somatosensory stimulus is best described as a vibration (vibe). Figure 3 shows the somatosensory stimulus

Figure 3

Response Measurement Details



Note. SES stands for "startle eliciting stimulus," ISI is the "inter-stimulus-interval", and ITI means "inter-trial-intervals."

(“vibe”), auditory stimulus (“aud”), and the physical response of the mouse through the accelerometer (“accel”). In the sound booth, the shaker creates the somatosensory vibration, a speaker makes the auditory stimulus, the accelerometer records the mouse’s responses, and a second speaker provides the “startle-eliciting-stimulus.”

Startle-Eliciting-Stimuli

In our research, we used a startle-eliciting-stimulus to evoke a startle response from the mice. A startle, or startle response, can best be described as “an extreme response to a highly novel and/or intense stimulus that carries potential major salience for the intact organism” (Blumenfeld & Faingold, 2014, p. 392). According to the researchers at James Madison University, “[Salience] is a simple measure of the extent to which the average mouse responded to the vibration” (Brett et al., 2021, Analyses section, para. 6). We then used this method to collect data on pre-pulse inhibition.

Pre-Pulse Inhibition

Researchers at Standford Medicine explain, “Pre-pulse inhibition is a phenomenon in which a weak stimulus (pre-pulse) can suppress the startle response to a subsequent stronger startle stimulus (pulse)” (Behavioral and Functional Research Laboratory, 2022, para. 1). For example, if there is a pattern of sounds- let’s call them “beeps”- that present as “high, low, high, low, high, low,” and then a startle-eliciting-stimulus (SES) is presented, the startle response is expected to be high. This is because the startle-eliciting-stimulus is unexpected. However, if these “beeps” presented are “high, low, high, low, *low, high,*” and then the startle-eliciting-stimulus is presented, the startle response will be lower. This is due to the pattern changing right before the SES is presented. When this happens, a part of the brain recognizes that because the

pattern changed suddenly, something else might change as well. Thus, the ability to startle less because of attention to pattern changes results in what is known as pre-pulse inhibition.

Application to the Research

According to Dr. Gabriele and Dr. Gray, "Variations of our novel multimodal pre-pulse inhibition (PPI) methods will serve as behavioral readouts of [the lateral cortex of the inferior colliculus] circuit functionality, as PPI processing runs through the LCIC and altered PPI and impairments in multisensory perceptual binding are associated with certain neurodevelopmental disorders, including autism" (2020, p. 11). This means that we can use pre-pulse inhibition as a way to measure data between the wild-type, heterozygous, and homozygous mice, thus giving us a way to measure if the mutation is significant. This either confirms or denies a link to neurodevelopmental disorders.

Hypotheses

The main prediction of the experiment is that the sum of the unimodal somatosensory and auditory pre-pulses will not be equal to the multimodal response in homozygous (mutant) mice. In simpler terms, the auditory stimulus and the vibration will not add up to the response that is multisensory. If we confirm our hypothesis, and "the mutant mice 'struggle' when confronted with cues requiring multisensory integration," then we can relate this to autistic individuals, as they "often miss the forest for the trees" (Gray, 2021, p. 6). Through our research, we can potentially find a genetic component of sensory processing and integration difficulties in autism spectrum disorder.

4. Results

Unimodal Somatosensory

After completing 172 trials (stimuli design shown in Figure 4), we found that the mutated mice had a significant interaction with the unimodal somatosensory vibration and PPI.

Figure 4

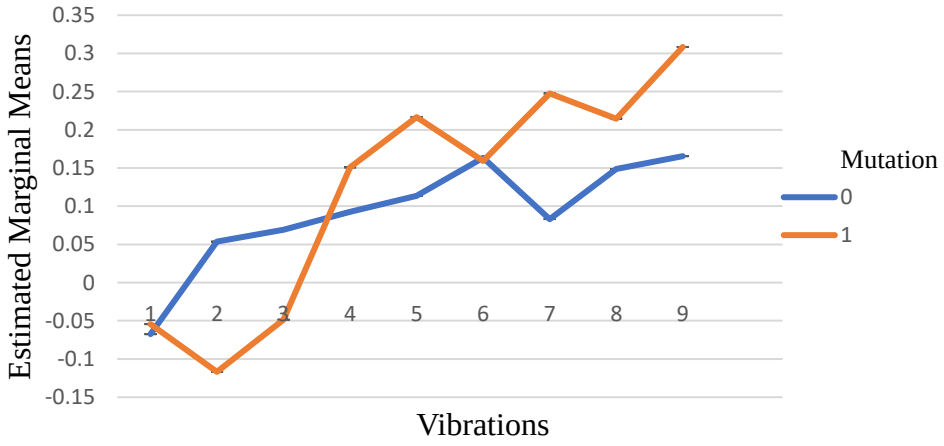
Stimuli Design

MODE	PURPOSE	PRE-PULSE	SES	PPI	TRIALS	WITHIN
1	Baseline	None	No	No	4	1
2	Somatosensory	None	No	No	24	12
3	Somatosensory Pre-Pulse	3 Hz- 2dB-2ms	Yes	Yes	48	12
4	Auditory Pre-Pulse	70dB; 10 or 20ms	Yes	Yes	8	2
5	Multimodal Pre-Pulse	Auditory and Somatosensory	Yes	Yes	48	12
6	Sound of Somatosensory	Sound of vibration	Yes	No	24	12
7	Startle Eliciting Stimulus	None	Yes	No	16	1
	Totals				172	52

Figure 5 shows that the mutation did affect the pre-pulse inhibition. Our research indicates a "highly significant interaction of mutation with the overall 'salience' of the vibrations (linear

Figure 5

Estimated Marginal Means of Somatosensory PPI



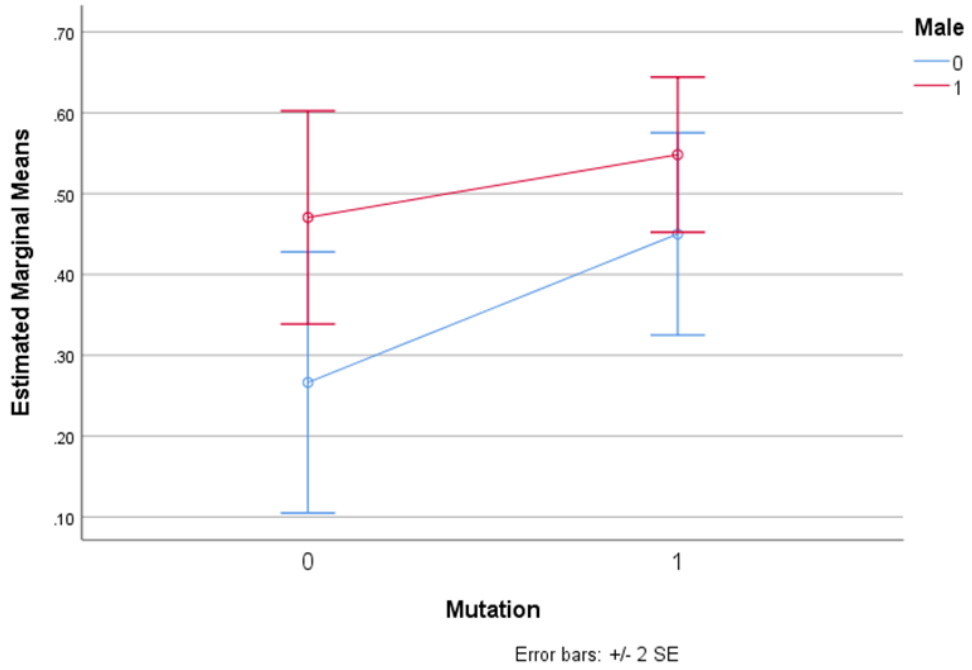
interaction. p=.007)" (Gray, 2021, p. 14). More specifically, this figure shows us that the homozygous mice had a greater growth of magnitude perception compared to the heterozygous mice and the wild-type mice.

Unimodal Auditory

For the unimodal auditory stimulus, Figure 6 shows that both the mutation and sex were significant. Other research studies have shown that sex does have an effect on pre-pulse

Figure 6

Estimated Marginal Means of Auditory PPI



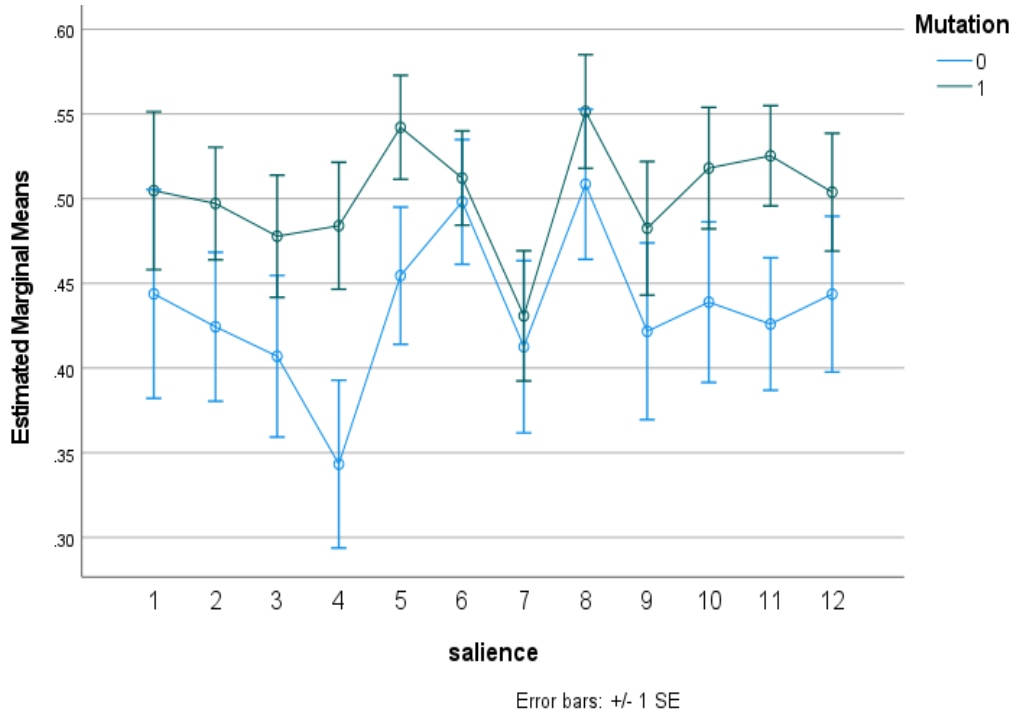
inhibition when it comes to auditory stimuli, as males displayed higher marginal means (Gómez-Nieto et al., 2020). This means that the male mouse unimodal auditory behavioral response replicated results from other published studies, and that the research is on the right track. However, something that sets this specific experiment apart is that the results also showed significance in the mutated mice, which means that the mutation did affect the unimodal auditory pre-pulse inhibition (Gray, 2021).

Multimodal (Somatosensory and Auditory)

When we analyzed the data for the multimodal behavioral responses, we found that the mutation did not have an effect on the pre-pulse inhibition. Figure 7 supports this claim.

Figure 7

Estimated Marginal Means of Multimodal PPI



Additionally, the data shows that there was almost significance for sex in the mutation (males greater than females), but it was not enough to be declared statistically significant (probability = .055 compared to p = .06).

Discussion

Overall, the data shows significance in the mutation's pre-pulse inhibition for the unimodal somatosensory and unimodal auditory behavioral stimuli. Also, sex in the mutation was found to be significant in both the auditory and somatosensory unimodal methods. However, when it came to the multimodal stimuli, there was no significance in the mutation (or for sex). If

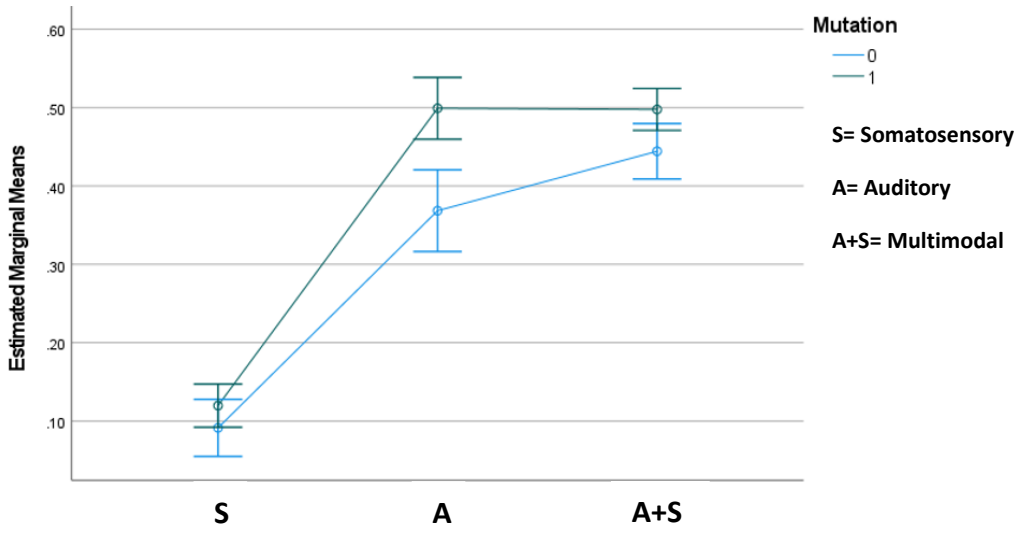
our data shows us that there was significance when it came to the unimodal stimuli (separately), why was there a lack of significance when the stimuli were presented simultaneously (multimodal)? Furthermore, does the sum of the results of the unimodal auditory and unimodal somatosensory stimuli equal the results of the multimodal behavioral response. In other words:

Does (A) + (S) = (A+S)?

For wild-type and heterozygous mice, this equation is true. But in the homozygous mice, the sum of the results of the unimodal auditory and unimodal somatosensory stimuli did not equal the

Figure 8

Estimated Marginal Means of PPI Across All Modes



results of the multimodal behavioral response, which supports our hypothesis. These outcomes are shown in Figure 8. According to Dr. Lincoln Gray, "Most importantly, [mutant] multimodal responsiveness is LESS than the sum of their unimodal responsiveness," which shows that the mutant mice did struggle with multisensory integration (Gray, 2021, p. 20).

5. Conclusion

Outcomes

The connection between this mutation in microglial cells and autism spectrum disorder means that we now have a deeper understanding of how the genetics of autism work. This discovery has to potential to influence the identification timeline, school accommodations, and future research. As speech-language pathologists know, earlier identification means earlier intervention. The goal is to start intervention as soon as possible because of the critical period and the need to have each individual equipped for success when they start attending school. An earlier diagnosis of a neurodevelopmental disorder could also give the caregivers piece of mind when it comes to their child's difficulties and/ or differences. A label could allow them to join support groups, research information, and process the diagnosis at an earlier stage, which may also decrease frustration and stress levels. When it comes to accommodations for autistic students in schools, it may be difficult to figure out where to start. With this research, we can focus on isolating multisensory integration. This can be through dimming lights, utilizing noise-cancelling headphones, or other techniques. While these accommodations are usually already seen in schools, we now know to address them first instead of testing out a number of other accommodations. This research has the potential to help everyone, not just autistic individuals. This biological basis for struggling with multisensory integration means that we can target a consequence that may come with it- divided attention. Teaching techniques for success while targeting attention can help a magnitude of individuals, including those with attention deficit/ hyperactivity disorder. Lastly, because our data shows significance, new studies can be conducted that can take an even deeper dive into these same compromised signals, which could then lead to an endless amount of new scientific discoveries.

Future Directions

My time in the Autism Certificate Program and Exceptional Education minor at James Madison University has equipped me with the knowledge and ability to conduct this research in a responsible and ethical manner. The goal is not to rid the genes that contribute to autism, but instead provide us with a better understanding of the genetic background. I plan to use all I have learned in my undergraduate research experience in my research-centered graduate assistanceship next year. With these skills, I plan to continue collecting data, drawing conclusions, and volunteering my time and efforts into making discoveries that have positive contributions to society.

6. Personal Reflection

Getting Started

On September 19th, 2020, I sent Dr. Lincoln Gray an email that was equivalent to a plea for help. It was the middle of the COVID-19 pandemic, and in anticipation for collecting research for my honors thesis, I wanted to start volunteering in a research lab for the Department of Communication Sciences and Disorders (CSD). Typically, an honors student in CSD volunteers in a research lab the spring semester of their junior year, collects research the fall of their senior year, and writes their thesis the spring of their senior year. I, however, was participating in an all-virtual course load and was eager to get some in-person and hands-on experience. So, I emailed the CSD honors liaison, Dr. Gray, the fall of my junior year. This way, I would be getting a head start, and would be able to volunteer for two semesters before I started planning and collecting data for my capstone. The email I received in response was funny, lighthearted, kind, and informative. I knew from his first email that Dr. Gray would be a great liaison and supporter throughout my honors project. He let me know that, currently, there were not any openings for a volunteer in faculty labs due to COVID-19 restrictions and existing spots filling up with honors students a year ahead of me. However, he did have another idea in mind. Dr. Gray informed me that he experiments in a mouse lab along with Dr. Mark Gabriele, a biology professor, and there are less COVID-19 restrictions because they aren't working with human participants. This left me with two choices- wait and try to get into a lab next semester, or take a tour of the mouse lab.

Touring the Laboratory

The next Thursday, I walked into Miller Hall, which houses some animal labs along with the planetarium. I met Dr. Gray and we walked down to the basement where the mouse lab is. He

swiped his JMU access card to get into the first set of doors. We then had to enter a code to get into the room where lab coats are kept. Next, we needed another code to get into the lab area. I was surprised by the number of codes there were, yet it made sense since important research was taking place and needed to be protected. There were two more codes we needed to enter, one for the office/ lab area, and one for where the mice were kept. I peeked at all the mice, and they were cuter than I had expected. I'd imagined that they were white with beady, red eyes, but they actually looked more like field mice. They were small, brown, and had pretty big ears. This eased my nerves, as I had never worked in a research lab before, much less an animal lab. Then, Dr. Gray showed me the equipment, testing area, and computer program. It was overwhelming at first, but I knew it was something I would eventually learn and become confident in. He explained that the experiments take place in a sound booth where a single mouse is placed in a horizontal tube and its responses are recorded with the equipment. The computer program tracks and graphs these responses as they happen. Depending on the type of experiment, each mouse is tested anywhere from 30 to 90 minutes. As the tour progressed, I became more and more eager to start volunteering with the mice, so I let Dr. Gray know that I was interested in the position.

Training

The next steps included lots of training and paperwork. I filled out a form that gave permission for my student information to be disclosed to the offices that needed it for the training, a health risk assessment form, completed a vivarium training, and fulfilled the Institutional Animal Care and Use Committee (IACUC) training requirement. The vivarium orientation modules consisted of a narrated PowerPoint presentation that students working in animal laboratories are required to go through and study. Then, we must take and pass a quiz on the material. The IACUC training was similar, as we were asked to read through modules of

information and then pass a series of quizzes. The National Institutes of Health (NIH) states that "the IACUC is responsible for oversight of the animal care and use program and its components" (Office of Laboratory Animal Welfare, 2022, Responsibilities section, para. 1). Like other institutions conducting animal research, James Madison University has its own Institutional Animal Care and Use Committee with members that are expected to monitor and oversee animal use. After many hours of reviewing material, studying procedures, and taking quizzes, I was officially ready to start in the mouse laboratory.

Experiences

In the very beginning, it was somewhat odd swiping my JMU access card, entering four codes on four different pin pads, and putting on a white lab coat every week. Yet, as time went on, I became more and more accustomed to it. The first few times in the lab, I met Dr. Gray so he could assist if I needed him to. Dr. Gray did a wonderful job of facilitating success, as he watched over my shoulder and let me independently turn on machines, enter data into the computer system, and "catch a mouse" to put into the tube. As I became more confident, I moved on to volunteer at the same time as a fellow undergraduate researcher to watch how they performed each task. After a couple of weeks of shadowing, I was ready to go to the lab by myself. By then, it was the start of the spring semester. The first week back from winter break, I put on a lab coat, took a deep breath, and got to work. I completed each task and gained a newfound sense of confidence and independence as each minute went by. It was truly hard to believe that I was doing research in a mouse laboratory at an institution, much less performing research for a project that was so generously funded by a grant from the National Institutes of Health. As each semester went on, I volunteered in, and talked about, the mouse lab as often as I could. Every Wednesday morning, I woke up excited to see the mice and conduct research. Plus,

Dr. Gray almost always had calming classical music playing in the lab, which acted as a useful tool to relieve stress. Just this year, I was able to convince two younger peers to start volunteering there as well. In closing, if you told me four years ago that I would be a research assistant working with mice in James Madison University's Computational, Speech, Sensory, Development & Diseases Lab, I truly never would have believed it.

References

Arnoux, I., & Audinat, E. (2014, August 4). *Fractalkine Signaling and Microglia Functions in the Developing Brain.* Retrieved from National Institutes of Health: https://pubmed.ncbi.nlm.nih.gov/26347402/#:~:text=Fractalkine%20signaling%20controls%20these%20functions,key%20player%20in%20neurodevelopmental%20disorders.

Behavioral and Functional Research Laboratory. (2022). *Prepulse Inhibition and Startle Reflex Test.* Retrieved from Stanford Medicine: https://med.stanford.edu/sbfnl/services/bm/ep/pre-pulse.html

Blumenfeld, H., & Faingold, C. L. (2014). *Neuronal Networks in Brain Function, CNS Disorders, and Therapeutics.* New York: Elsevier Inc.

Brett, C., Gabriele, M., & Gray, L. (2021). Fractalkine Signaling Affects Behavioral Responses to Sounds and Vibrations in Mice. Retrieved April 9, 2021

Brown, L. (2011, November). *Identity-First Language.* Retrieved from Autistic Self Advocacy Network: https://autisticadvocacy.org/about-asan/identity-first-language/

Feinberg School of Medicine Research Office. (2018, March). *Understanding the Biology of Autism.* Retrieved from Northwestern Medicine: https://www.feinberg.northwestern.edu/research/news/podcast/biology-of-autism.html

Gabriele, M. (2020, September 16). *Role of Microglia in Sculpting Multisensory Midbrain Circuits.* Retrieved from National Institutes of Health: https://reporter.nih.gov/project-details/10041307

Gabriele, M., & Gray, L. (2020). National Institutes of Health Grant Proposal. Harrisonburg, Virginia, United States of America. Retrieved April 9, 2021

Gabriele, M., & Gray, L. (2020). *Response Measurement Details* [Figure 3]. National Institutes of Health Grant Proposal. https://reporter.nih.gov/project-details/10041307

Gabriele, M., & Gray, L. (2020). *Schematics of Multimodal Behavioral Setup* [Figure 2]. National Institutes of Health Grant Proposal. https://reporter.nih.gov/project-details/10041307

Gabriele, M., Gray, L., & Shelton, G. (2021, October 20). Microglial Mutation Affects Auditory and Somatosensory Perceptions. Harrisonburg, Virginia, United States of America.

Gómez-Nieto, R., Hormigo, S., & López, D. E. (2020, September 10). *Prepulse Inhibition of the Auditory Startle Reflex Assessment as a Hallmark of Brainstem Sensorimotor Gating Mechanisms.* Retrieved from National Institutes of Health National Library of Medicine: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7563436/

Grandin, T. (2012). *Different... Not Less: Inspiring Stories of Achievement and Successful Employment from Adults with Autism, Asperger's, and ADHD.* Retrieved from Temple Grandin Ph.D.: https://www.templegrandin.com/books.html

Gray, L. (2021, April 6). Behavioral Effects of Microglial Cell Mutations. *Toward the Goals of the Gabriele and Gray, 2020-23 NIH Grant*. Department of Communication Sciences and Disorders, James Madison University.

Gray, Lincoln (2021). *Estimated Marginal Means of Auditory PPI* [Figure 6]. Behavioral Effects of Microglial Cell Mutations. *Toward the Goals of the Gabriele and Gray, 2020-23 NIH Grant*. Department of Communication Sciences and Disorders, James Madison University.

Gray, Lincoln (2021). *Estimated Marginal Means of Multimodal PPI* [Figure 7]. Behavioral Effects of Microglial Cell Mutations. *Toward the Goals of the Gabriele and Gray,*

2020-23 NIH Grant. Department of Communication Sciences and Disorders, James Madison University.

Gray, Lincoln (2021). *Estimated Marginal Means of PPI Across All Modes* [Figure 8]. Behavioral Effects of Microglial Cell Mutations. *Toward the Goals of the Gabriele and Gray, 2020-23 NIH Grant*. Department of Communication Sciences and Disorders, James Madison University.

Gray, Lincoln (2021). *Estimated Marginal Means of Somatosensory PPI* [Figure 5]. Behavioral Effects of Microglial Cell Mutations. *Toward the Goals of the Gabriele and Gray, 2020-23 NIH Grant*. Department of Communication Sciences and Disorders, James Madison University.

Gray, Lincoln (2021). *Stimuli Design* [Figure 4]. Behavioral Effects of Microglial Cell Mutations. *Toward the Goals of the Gabriele and Gray, 2020-23 NIH Grant*. Department of Communication Sciences and Disorders, James Madison University.

Intramural Research Program. (2014). *Understanding Sensory Symptoms in Autism: Seeing the Trees, but Not the Forest.* Retrieved from National Institutes of Health: https://irp.nih.gov/accomplishments/understanding-sensory-symptoms-in-autism-seeing-the-trees-but-not-the-forest#:~:text=People%20with%20 autism%20report%20 exceptionally ,neurobiological%20roots%20of%20autistic%20sensory

Johns Hopkins University. (2017, February 27). *What Experts Know- and Don't Know- About Autism and its Causes.* Retrieved from Johns Hopkins Hub: https://hub.jhu.edu/2017/02/27/autism-cause-treatment/

Kennedy Krieger Institute. (2019). *Cognitive Theories Explaining ASD.* Retrieved from Interactive Autism Network: https://iancommunity.org/cs/understanding_research/cognitive_theories_explaining_asds

Kim, S. H., & Lord, C. (2010). Restricted and Repetitive Behaviors in Toddlers and Preschoolers with Autism Spectrum Disorders Based on the Autism Diagnostic Observation Schedule. *Autism Research, 3,* 162-173. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3005305/

Magro, K. (2010, January 15). *Autism Can't Define Me; I Define Autism.* Retrieved from Kerry Magro: https://kerrymagro.com/autism-cant-define-me-i-define-autism/

MathWorks. (2022). *What is MATLAB?* Retrieved from MathWorks: https://www.mathworks.com/discovery/what-is-matlab.html

National Center on Birth Defects and Developmental Disabilities. (2021, March 29). *Signs and Symptoms of Autism Spectrum Disorders.* Retrieved from Centers for Disease Control and Prevention: https://www.cdc.gov/ncbddd/autism/signs.html

National Human Genome Research Institute (2022). Heterozygous and Homozygous Genotypes [Figure 1]. National Institutes of Health. https://www.genome.gov/genetics-glossary/heterozygous

National Institute of Mental Health. (2022, March). *Autism Spectrum Disorder.* Retrieved from National Institutes of Health: https://www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd

Office of Laboratory Animal Welfare. (2022). *The Institutional Animal Care and Use Committee.* Retrieved from National Institutes of Health: https://olaw.nih.gov/resources/tutorial/iacuc.htm

Sibley, K. (2012, October 4). *What is an Ally?* Retrieved from Autistic Slef Advocacy Network: https://autisticadvocacy.org/2012/10/what-is-an-ally/

Scott-Hewitt, N. (2020). *Immune 'Eat-Me' Signal Contributes to Synaptic Pruning During Development.* Retrieved from Harvard Brain Science Initiative: https://brain.harvard.edu/hbi_news/immune-eat-me-signal-contributes-to-synaptic-pruning-during-development/

Shore, S. (2018). *Interview with Dr. Stephen Shore: Autism Advocate & on the Spectrum.* Retrieved from International Board of Credentialing and Continuing Education Standards: https://ibcces.org/blog/2018/03/23/12748/