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Weber State University Bachelor of Integrated Studies Program

Mackenzie Margulies Moon

Date: 7/15/2021

Project Title: Validation of Behaviors in a Zebrafish Model of Autism

Brief summary of project: The cause of autism is multifactorial but has a strong genetic component dependent on the expression of certain genes. When these genes are incorrectly expressed the process of synaptogenesis may be disrupted, causing behavioral deficits to manifest. The following primary research project involves exposing wildtype zebrafish embryos to valproic acid (VPA) to induce autistic characteristics and behaviors. VPA is also known to alter expression of genes involved in autism. The altered expression of ASD-related genes on its own does not show the whole picture. The model must be tested behaviorally to tie the behavioral deficits to the gene being tested. The genetic and behavioral aspects of autism research each have their own pieces that together allow a greater understanding of the puzzle of autism.

Area of Emphasis 1: Neuroscience

Committee Member from that discipline: Dr. Elizabeth Sandquist

Area of Emphasis 2: Psychology

Committee Member from this discipline: Seth Wilhelmsen

Area of Emphasis 3: Health Sciences

Committee Member from this discipline: Dr. Jim Hutchins

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Nature of the Problem

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder marked by social and communication deficits as well as repetitive motor behaviors known as stimming. The symptoms of ASD usually manifest in late infancy and early childhood. The cause of autism is multifactorial but has a strong genetic component dependent on the expression of certain genes. When these genes are incorrectly expressed the process of synaptogenesis may be disrupted, causing behavioral deficits to manifest (Kozol, 2018). Social deficits, difficulty with communication, and repetitive behaviors are defining characteristics of ASD, but these do not always manifest the same in each case. ASD is a rainbow of behaviors and abilities with differing degrees of severity. Other symptoms associated with autism include anxiety, inattentiveness, irritability, and low cognitive function (Fernell et al., 2013).

ASD is typically diagnosed by an individual's pediatrician or a psychiatrist. Diagnostic tests used to determine whether a patient has ASD include the Screening Tool for Autism in Toddlers and Young Children (STAT), the Autism Diagnostic Observation Schedule (ADOS), and the Autism Diagnostic Interview-Revised (ADI-R), which interviews the patient's caregiver to seek more information about observed behaviors and family history (Lord, et. al., 2018). The most well-known and commonly used diagnostic test is the ADOS.

According to the Centers for Disease Control, which began tracking Autism cases in 1998, the prevalence of autism spectrum disorder in the United States has increased from 1-in-150 to 1-in-54 children (Maenner 2020). This increase may be a result of earlier intervention and a major

change in diagnostic criteria in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5. Prior to 2013, Autism, Asperger's Syndrome, and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), were considered separate disorders (Tsai & Ghaziuddin). The DSM-5 released in 2013 merged these disorders into a single diagnosis of autism spectrum disorder.

There are currently no therapeutic pharmaceutical interventions intended specifically for treatment of ASD, but health care providers frequently prescribe medications to treat conditions associated with ASD such as anxiety or ADHD. Most often, treatment comes in the form of educational and behavioral interventions such as Applied Behavior Analysis (ABA). The details of intervention for each individual client are laid out and supervised by clinicians holding the title of Board-Certified Behavior Analyst and implemented by Registered Behavior Technicians.

Behaviors Associated with ASD

Autism spectrum disorder presents differently in each case, but there are core behaviors that are seen among individuals on the spectrum. According to the DSM-5, diagnosis for ASD is based on two sets of criteria: Persistent deficits in social communication and interaction, as well as restrictive, repetitive sensory-motor behaviors. Deficits in social communication may include failure to initiate interaction with peers or respond appropriately to peer interactions, difficulty creating and maintaining intrapersonal relationships, and deficits in nonverbal communication. Nonverbal communication deficiencies often present as lack of facial expressions, too much or too little eye contact, and difficulty understanding body language and social cues. These deficits usually show up early in life. Restrictive, repetitive sensory-motor behaviors may include repetitive motor movements such as hand-flapping, spinning, or toe wiggling. These motor movements are commonly referred to as "stimming". Other items included in these criteria

include echolalia, which is repetition of speech or sounds, cognitive inflexibility, and abnormal fixative interests (American Psychiatric Association, 2013). Other commonly associated behaviors include aggression, anxiety, and hyperactivity. The severity and frequency of behaviors associated with ASD are taken into consideration for diagnosis.

Screening and Diagnosis

The CDC began tracking ASD in 1996 and established the Autism and Developmental Disabilities Monitoring (ADDM) Network in 2000. The ADDM network is tasked with tracking autism prevalence among 8-year-old children at sites across the United States. The most recent results from 2016 found that 1-in-54 children of 8 years of age have ASD. The study also found that boys were 4.3 times more likely to be diagnosed with ASD than girls. The data collected from the ADDM Network is intended to be used to provide appropriate levels of community support and improve early diagnosis and intervention rates (Maenner et. al., 2016).

Early diagnosis of ASD and subsequent early intervention are important to the success of treatment and quality of life for individuals on the spectrum. As discussed in the previous section, autism diagnosis is predicated upon deficits in social communication and the presence of restrictive, repetitive motor behaviors. These criteria are listed in the DSM-5 under the single diagnosis of autism spectrum disorder. While the DSM-5 provides a checklist of criteria to be satisfied for the diagnosis of ASD, a variety of other screening and diagnostic tools are available to healthcare professionals and behavioral specialists. A common screening tool used by pediatricians is the Modified Checklist for Autism in Toddlers (M-CHAT), and less commonly, the Communication and Symbolic Behavior Scales (CSBS). Standardized diagnostic tools such as the Autism Diagnostic Observation Schedule (ADOS) and (STAT) are the next step in potentially diagnosing ASD. The ADOS, considered the Gold Standard for diagnostic tools, is a

45-minute behavior screening available in age-appropriate modules from one year of age through adulthood. STAT is a shorter, 20-minute screening targeted exclusively to young children (Lord et al., 2018). Levels of severity range from level 1 to level 3, depending on the amount of intervention required. Level 1 ASD is defined as 'requiring some support', Level 2 requires 'substantial support', and Level 3 requires 'very substantial support'.

Treatment

Once a diagnosis is in place, treatment usually comes in the form of early naturalistic developmental behavioral interventions such as Applied Behavioral Analysis (ABA). ABA is a scientific approach to behavior modification that identifies meaningful variables to reinforce the learning of desired behaviors while decreasing the frequency and intensity of undesirable behaviors (Dyer, 2013). The programs used for individual clients are put in place by skilled professionals known as Board Certified Behavior Analysts, though they are typically implemented by registered behavior technicians who work directly with the client. Depending on need, clients typically receive 15 or more hours a week of intervention.

Pharmaceutical intervention may be used to treat symptoms like anxiety, or comorbidities such as depressive disorders or ADHD. These medications include SSRIs and stimulants, but these are not FDA approved to specifically treat symptoms of ASD. The only medication currently approved by the FDA for this purpose is an atypical antipsychotic by the name of Risperidone, which is prescribed to treat irritability (Lord et. al., 2018).

Genes Associated with ASD

One common factor in the etiology of ASD is synaptogenesis. Synaptogenesis is the creation of synapses between neurons, allowing the cells to communicate with one another. This process begins around 5 weeks gestation in humans and continues for the first two years

postnatally, though mild synaptogenesis occurs throughout life (Budday et al., 2015). The process of synaptogenesis during development is dependent on the expression of certain genes. Genes strongly associated with ASD and disruption of synaptogenesis include SYNGAP1, NRXN1, NGLN3, and SHANK3 (Kozol at al., 2013; Fernell et al., 2013; Dwivedi et al., 2019) which have been studied using a zebrafish model. Current research of these genes is being performed at Weber State University by Brittney Child under the direction of Dr. Elizabeth Sandquist and Dr. Jim Hutchins. The genes under investigation include PTCH2 and LMX1bb.

ZEBRAFISH MODELS OF ASD

History

The use of zebrafish (*Danio rerio*) in biomedical research was established by Dr. George Streisinger in the 1970s. Streisinger engineered the first zebrafish genetic mutant, *golden*, during his tenure at the University of Oregon (Nüsslein-Volhard & Dahm, 2002). 40 years later there are now over 117,000 mutant fish listed in the Zebrafish Information Network (ZFIN) database (2021).

Benefits of a Zebrafish Model in Biomedical and Behavioral Research

Unlike rodents, fertilization and embryonic development are ex vivo, meaning they occur externally. An adult pair of zebrafish can produce 200 to 300 eggs per mating session, with embryos that are completely transparent for the first 24 hours post-fertilization (hpf) (Lee et al., 2018). Another advantage to a zebrafish model is the short maturation period. Embryonic development is complete within 72 hours post-fertilization with synaptogenesis beginning around 16-hpf. The larval stage spans from 3 days post-fertilization (dpf) to 30-dpf. Fish reach sexual maturity by 90-dpf (Nüsslein-Volhard & Dahm, 2002). Zebrafish are social vertebrates that prefer swimming in groups, an activity called shoaling, and display an even greater

propensity for social interaction than traditional rodent models, making them ideal for observing social behaviors (Saverino & Gerlai, 2008). In addition to these observational advantages, the gene sequences of zebrafish and humans are approximately 70% homologous, and 84% of genes associated with human disease, including those associated with autism, have zebrafish counterparts (Kaleuff et al., 2014).

ZEBRAFISH MODEL OF ASD AT WEBER STATE UNIVERSITY

1. Introduction

As the mother of three children on the autism spectrum, this project was very personal and close to my heart. From the time my children were diagnosed with ASD, I knew I wanted to go into the field of autism research. As my neuroscience professor, Dr. Jim Hutchins recognized my interest and encouraged me to participate in autism research at Weber State University. In preparation for this primary research, I began studying the zebrafish model of autism in January 2020 with Dr. Hutchins and Brittney Child. Our small journal club met weekly to discuss academic articles pertaining to zebrafish research. March 2020 saw the rise of COVID-19 as the world went into isolation, and our journal club temporarily adjourned. Despite the continuation of the pandemic our meetings resumed via Zoom during Summer 2020 and grew to include the expertise of Dr. Elizabeth Sandquist and a new group of interested students. By the end of the summer semester I had the honor of joining Dr. Sandquist's lab to participate in the care and husbandry of the zebrafish while I prepared to conduct my research and behavioral tests. My responsibilities in the lab included preparing brine, feeding adult fish and larva, assisting with mating adult fish, and training new members of the lab. During this time, I had the opportunity to write an undergraduate research grant proposal requesting funds from the Weber State University Office of Undergraduate Research. Hundreds of hours went into writing and revising

the proposal, but the hard work paid off and the grant was funded in November 2020. Collection of test animals and behavior assays commenced in spring 2021.

Investigation of genes associated with ASD is essential to understanding the potential causes and indications of this disorder, but gene expression is only one part of the picture. Behaviors associated with ASD must also be present. Previous research has been performed to determine if zebrafish treated with valproic acid display behaviors analogous to humans with autism spectrum disorder. Replication of this research is necessary to validate observable behaviors in a zebrafish model of autism. It is also essential to establish a behavioral baseline against which future researchers may compare specimens that express a candidate gene. Based on previous research of the effects of VPA on behavior, I hypothesize that the group treated with VPA will display significantly higher rates of behaviors associated with ASD than the control group.

2. Materials and Methods

2.1 Animals

Male and female adult wildtype zebrafish were placed in mating tanks and separated by dividers overnight. Dividers were pulled the following morning and embryos were collected within 15 minutes of fertilization and separated into two groups: Control and experimental. Experimental embryos were placed in a standard sized petri dish containing 30 mL of embryo medium treated with 50 μM of valproic acid (VPA) for the first 48-hours post-fertilization (n = 50). Control fish were maintained in 30 mL of untreated embryo medium. The concentration and duration of VPA exposure were chosen based on research performed by Zimmerman et al. (2015). The number of embryos and size of the dish were chosen based on established animal husbandry practices in the lab. At 48 hours control and experimental fish were moved to fresh

untreated embryo medium until 7 days post-fertilization. At that time, the larvae were moved to and maintained in an Aquaneering Zebrafish Aquatic Housing System.

2.2. Behavioral Assessments

Thigmotaxis and open-field behavior assessments and social preference assessments were based on behavioral assays highlighted in the article *Embryological exposure to valproic acid induces social interaction deficits in zebrafish (Danio rerio): A developmental behavior analysis* by FF Zimmerman et al. (2015). Inattentive behaviors were assessed based on the research of Shubham Dwivedi et al. in the *article Larval zebrafish model for studying the effects of valproic acid on neurodevelopment: An approach towards modeling autism*. All tests were recorded using a Sony HDRCX405 HD camcorder and analyzed using ANY-maze Video Tracking System software.

2.2.1 Thigmotaxis

Thigmotaxis (wall-sticking behavior) is a known indicator of anxiety in zebrafish (Kalueff et al., 2014). To test this behavior, 6-dpf larvae were individually placed in the wells of a 24 well plate filled with 3 ml of E3 media. Each well measured a diameter of 16 mm. Plates were placed in a dark room on a light box with a single sheet of copy paper to decrease light intensity. Larvae were recorded for 5 minutes following 1 minute of acclimation. Locomotion was verified using total distance travelled. Thigmotaxis was evaluated by the number of entries into the outer zone, which was designated as the first 3 mm from the edge of the well. The total number of seconds spent in the outer zone was also evaluated.

2.2.3. Locomotion

Locomotor activity was measured at 30 and 60 days post-fertilization in a room with a light intensity measuring 264 LUX. Animals were placed in test tanks measuring 24.8 cm x 9.6 cm x 15.9cm filled with 1030 mL of system water and recorded for 5 minutes following a 1 minute acclimation period. The tank was divided into two equal horizontal sections and 4 equal vertical sections. Time spent in each horizontal section was an index of anxiety, with a greater amount of time in the lower section indicating a greater level of anxiety. Locomotion was evaluated using distance travelled, mean speed, and the number of combined vertical and horizontal line crossings.

2.2.3 Social Preference

Social preference was measured at 70-dpf by placing individual fish in a central tank measuring 24.8 cm x 9.6 cm x 15.9cm filled with 1030 mL of system water. An empty tank was positioned directly to the left, and a social tank containing 15 stimulus fish directly to the right. The tanks were placed in contact with one another allowing the test fish to see into either tank. The central test tank was divided in an identical manner to the 30-dpf and 60-dpf locomotion tests with one horizontal line separating equal upper and lower sections, and 4 vertical lines creating 4 equal vertical sections. The light intensity of the room where testing occurred measured 264 LUX. Behaviors were recorded for 5 minutes following a 1-minute acclimation period. Social preference was evaluated by the amount of time spent in the zone closest to the stimulus tank (Zimmerman, 2015). Locomotion was evaluated using distance travelled, mean speed, and the number of combined vertical and horizontal line crossings.

2.2.4 Inattentive Behavior

Inattentive behavior was measured at 7-dpf. 150 mL agarose gel (2% in E3 media) was poured into a crossing tank measuring 19cm x 8cm x 7cm. Four lanes measuring 75mm x 25mm x 5mm were carved out using glass microscope slides as a template. E3 media was poured into the lanes of the cooled gel and the tank was placed atop a 7-inch Kindle screen playing a 30-minute MP4 video of white background with a moving red line on the lower half of the screen. The number of larvae placed in each lane was n = 10. The tanks were recorded from above for 30 minutes following 30 seconds of acclimation. The number of larvae in the upper section of the tank were counted every two minutes and the average of each test was taken.   Inattentive behavior was determined by the number of larvae which avoided the lower half (Dwivedi et al., 2019).. The percentage of larvae that avoided the aversive stimulus was determined by the equation

$$\left(\frac{Aversive\ Stimulus - Acclimation}{Acclimation}\right) * 100$$

.

3. Results

3.1 Thigmotaxis Results

Contrary to our hypothesis, a one-way ANOVA of thigmotaxis at 6-dpf found no statistically significant difference between the VPA group (n = 81, M = 11.16, SD = 8.75) and the control group regarding the number of entries in the outer zone. There was also no significant difference between the amount of time spent in the outer zone in the VPA group of fish (n = 81, M = 238.24, SD = 49.02) and the control group (n = 74, M = 235.68, SD = 59.74). The results of this

test indicates the difference in the levels of anxiety between the two groups was not statistically significant. There were also no statistically significant differences between the rates of locomotion in the VPA group (n = 81, M = 0.38, SD = 0.26) and the control group (n = 74, M = 0.38, SD = 0.23).

3.2 *Locomotion Results*

Analysis of 30-dpf locomotion showed that the VPA group spent significantly more time in bottom zone of the tank than the control group (p < .05). This indicates a greater level of anxiety in the VPA group, t = 2.0699, df = 30, standard error of difference = 16.44. The differences in distance travelled, average speed, or number of line crossings between the two groups were not found to be statistically significant. 60-dpf locomotion tests did not display any statistically significant differences between groups in time spent in the bottom zone, distance travelled, average speed, or number of line crossings (see Table 1). VPA group displayed higher levels of anxiety at the 30-day timepoint than at the 60-day timepoint, possibly indicating that anxiety levels decreased as the fish matured.

Age	Anxiety Parameter	Control group Means ± SD	n	VPA group Means ± SD	n	p-value
6-dpf	Entries in the outer zone	9.4 ± 5.4	74	11.2 ± 8.7	81	p > .05
6-dpf	Time spent in outer zone	235.7 ± 59.7	74	238.2 ± 49.0	81	p > .05
30-dpf	Time spent in bottom zone	178.7 ± 47.7 s	18	212.7 ± 44.0 s	14	p < .05
60-dpf	Time spent in bottom zone	129.7 ± 65.9 s	12	159.7 ± 38.2 s	15	p > .05

Table 1: Anxiety parameters for VPA-treated and control groups at different ages.

3.2 *Social Preference Results*

The social preference testing performed at 70-dpf assessed time spent in each of the 4 zones, total distance travelled, average speed, and number of line crossings. Contrary to our hypothesis

and results of previously published research, there was no statistically significant difference between groups in any of the listed parameters.

70 dpf Social Preference
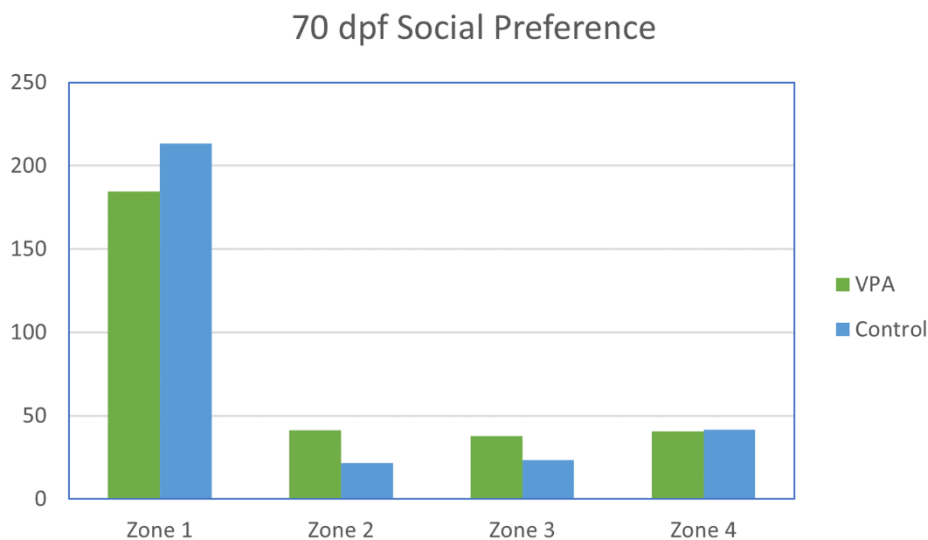
3.4 Inattentive Behavior Results

The mean percentage of VPA larvae in the upper zone increased by 33.3% while the number in the control group increased by 17.4%. A two-way ANOVA found that there was no statistically significant effect of VPA on the percentage of larvae avoiding the aversive stimulus $F(1,1) = 0.08922$, $p = 0.8152$. It should be noted that while the test was performed on two separate occasions, the raw data from the first test was accidentally deleted. It is also important to note that the crossing tank used to perform the test had the brand name visibly embossed on the bottom, a factor that may have affected results. With these factors in mind, the results of this test were inconclusive.

Discussion and Recommendations

Previous literature performing behavioral assays on fish treated with valproic acid yielded significant results, while this research did not. A noted difference between this research and

previously published research is in the administration of VPA to test embryos. In Zimmerman's research VPA was administered to groups of 15 embryos per well in 12 mL of VPA, while our research administered VPA to groups of approximately 50 or more embryos in 30 mL of VPA solution. Perhaps a greater number of fish per dish decreases the effects of the VPA. Future research may benefit from administration of a higher concentration of VPA, or adjustments in group size and ratio of embryo media to VPA.

I recommend at least two full semesters for animal husbandry and performance of tests. I was only able to reach the 70-dpf timepoint due to impending graduation, but continuing behavior tests to 120-dpf, or adulthood for the zebrafish, would provide greater insight into behavioral deficits through maturity. I recommend allowing enough time to replicate each behavioral test at least twice to increase the sample size as well as the validity of the results. If possible, younger stock fish should be utilized for producing test embryos, as it may yield a greater number of embryos with a higher fertilization and survival rate. Survival rates should be recorded and compared to look for potential differences, Future research may also benefit from analyzing data with software designed specifically for zebrafish research. ANY-maze software was originally designed for analysis of rodent behavioral assays. The software experiences delays or crashes when analyzing more than 12 subjects per test. As a result, it may not ideal for analyzing zebrafish research. For behavioral tests such as those performed at 6-dpf and 7-dpf, a camera apparatus that allows the camera to be positioned directly above the test plates may allow for greater accuracy during analysis.

Despite nearly every test yielding results that were not statistically significant, I consider this project to be a success. I made many mistakes and suffered several setbacks along the way, but I learned so much more from my failures than I would have if everything had gone according to

plan. The wonderful thing about science is that failure generates knowledge, so no study is ever a true failure. I believe everything I have learned in the five semesters I have spent on this research will be invaluable to my graduate school experience and carry on into my future career. Through this project I have gained a new confidence in my abilities and have formed relationships with my peers and mentors that will last a lifetime. I am grateful to my committee members, the BIS department and the Office of Undergraduate Research for facilitating this life-changing experience.

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Appendix

I.

6-DPF TEST RESULTS AND STATISTICS

- **Control**
    - **Time (s) in the Outer Zone:** 291.2, 282.5, 277.3, 282.1, 280.8, 272.1, 291.7, 277.6, 273.2, 275.7, 181.1, 238.0, 273.9, 262.3, 264.1, 255.0, 299.5, 282.0, 159.1, 248.9, 226.3, 205.0, 246.9, 300.0, 276.9, 98.9, 282.0, 272.1, 230.6, 300.0, 288.1, 164.5, 288.6, 289.8, 204.1, 231.0, 208.4, 252.9, 94.3, 266.6, 280.6, 117.1, 276.2, 262.2, 178.7, 208.4, 232.3, 100.2, 129.4, 284.5, 295.2, 204.2, 293.9, 295.1, 179.1, 272.0, 238.8, 143.4, 266.4, 243.8, 260.6, 226.7, 246.6, 246.8, 226.0, 205.0, 214.0, 183.7, 179.6, 52.6, 278.0, 268.4, 77.2, 278.6,
        - $n = 74$, $M = 235.7$, $SD = 59.7$, sum = 17440.4

    - **Entries in the Outer Zone:** 6, 8, 4, 10, 9, 4, 4, 10, 11, 8, 23, 17, 7, 19, 12, 13, 2, 7, 16, 16, 16, 5, 12, 1, 6, 20, 1, 6, 5, 1, 3, 10, 5, 1, 11, 19, 8, 8, 6, 3, 8, 17, 7, 12, 8, 15, 17, 5, 6, 6, 3, 12, 3, 4, 23, 11, 9, 11, 12, 8, 8, 10, 10, 9, 8, 12, 8, 14, 24, 3, 12, 9, 8, 7
        - $n = 74$, $M = 9.4$, $SD = 5.4$

    - **Distance Travelled (m):** 0.558, 0.613, 0.080, 0.537, 0.371, 0.061, 0.281, 0.376, 0.535, 0.462, 0.355, 0.462, 0.132, 0.595, 0.313, 0.301, 0.138, 0.215, 0.277, 0.375, 0.698, 0.072, 0.609, 0.053, 0.282, 0.477, 0.052, 0.285, 0.210, 0.012, 0.500, 0.259, 0.424, 0.031, 0.161, 0.573, 0.200, 0.470, 0.088, 0.078, 0.485, 0.362, 0.584, 0.572, 0.091, 0.402, 1.000, 0.011, 0.161, 0.842, 0.339, 0.310, 0.747, 0.808, 0.335, 0.491, 0.180, 0.190, 0.207, 0.290, 0.647, 0.510, 0.708, 0.747, 0.326, 0.513, 0.666, 0.419, 0.640, 0.052, 0.626, 0.627, 0.082, 0.228
        - $n = 74$, $M = 0.38$, $SD = 0.23$, sum = 27.77

- **VPA**
    - **Time (s) in the Outer Zone:** 235.0, 295.7, 251.6, 240.6, 253.4, 177.3, 195.2, 185.2, 279.4, 297.4, 160.0, 256.5, 300.0, 287.0, 299.1, 300.0, 279.1, 262.6, 241.4, 246.8, 247.2, 131.0, 273.4, 273.0, 269.8, 205.0, 185.9, 292.7, 161.4, 292.6, 137.0, 98.9, 272.0, 278.3, 295.3, 228.4, 240.9, 262.9, 282.2, 278.1, 247.4, 197.7, 210.5, 297.3, 101.2, 284.0, 244.2, 195.7, 230.3, 202.6, 192.7, 230.7, 180.9, 202.6, 300.0, 234.7, 214.1, 257.2, 294.0, 183.0, 264.6, 245.7, 260.3, 289.5, 208.0, 281.9, 268.3, 239.5, 228.2, 254.6, 275.8, 225.1, 275.8, 232.2, 131.4, 158.1, 272.9, 180.2, 261.0, 239.2, 255.2
        - $n = 81$, $M = 238.2$, $SD = 49.0$, sum = 19297.6

    - **Entries in the Outer Zone:** 3, 2, 14, 19, 3, 10, 19, 4, 4, 3, 23, 20, 1, 11, 2, 1, 6, 17, 6, 21, 22, 32, 12, 17, 24, 12, 13, 10, 10, 6, 52, 6, 21, 12, 2, 11, 9, 9, 13, 11, 19, 9, 31, 2, 4, 3, 1, 2, 13, 29, 25, 3, 10, 16, 1, 15, 7, 14, 3, 6, 10, 10, 15, 5, 8, 2, 9, 10, 6, 8, 5, 12, 8, 21, 2, 21, 9, 9, 6, 10, 12
        - $n = 81$, $M = 11.2$, $SD = 8.8$

- **Distance Travelled (m):** 0.118, 0.168, 0.416, 0.341, 0.085, 0.110, 0.290, 0.186, 0.124, 0.076, 0.298, 0.619, 0.014, 0.544, 0.329, 0.026, 0.255, 0.411, 0.181, 0.544, 0.691, 0.157, 0.842, 0.641, 0.662, 0.163, 0.165, 0.697, 0.174, 0.769, 0.185, 0.187, 1.162, 0.625, 0.114, 0.413, 0.309, 0.339, 0.644, 0.733, 0.362, 0.201, 0.433, 0.896, 0.065, 0.085, 0.0 16, 0.029, 0.757, 0.585, 0.577, 0.257, 0.229, 0.231, 0.017, 0.467, 0.326, 0.370, 0.037, 0.076, 0.645, 0.244, 0.278, 0.505, 0.053, 0.049, 1.009, 0.555, 0.706, 0.710, 0.432, 0.480, 0.483, 0.638, 0.049, 0.499, 0.605, 0.373, 0.444, 0.543, 0.624
  - **n = 81, M = 0.38, SD = 0.26, sum = 30.75**

- **Results:**
  - **Time (s) in the Outer Zone:** The two-tailed P value equals 0.7701. By conventional criteria, the difference in thigmotaxis between the VPA and control group is considered to be **not statistically significant**.

  - **Entries in the Outer Zone:** The two-tailed P value equals 0.7684. By conventional criteria, this difference is considered to be **not statistically significant.**

  - **Distance Travelled (m):** The two-tailed P value equals 0.900. By conventional criteria, this difference of total distance travelled between VPA and control groups is considered to be **not statistically significant**.

LOCOMOTION TEST RESULTS AND STATISTICS

- **30-dpf Locomotion**
  - **Control**
    - **Time (s) in the bottom zone:** 130.0, 198.3, 108.0, 203.1, 151.5, 187.1, 205.5, 281.2, 171.2, 273.0, 122.0, 176.1, 193.8, 161.2, 127.1, 164.0, 218.5, 144.5
      - **n = 18, M = 178.7, SD = 47.7**

    - **Distance travelled (m):** 2.246, 2.993, 3.876, 12.163, 7.449, 3.450, 3.603, 2.875, 4.382, 0.876, 4.608, 8.584, 5.853, 4.466, 8.800, 6.597, 7.000, 7.021
      - **n = 18, M = 5.38, SD = 2.8**

    - **Average Speed (m/s):** 0.007, 0.010, 0.013, 0.041, 0.025, 0.012, 0.012, 0.010, 0.015, 0.003, 0.015, 0.029, 0.020, 0.015, 0.029, 0.022, 0.023, 0.023
      - **n = 18, M = 0.018, SD = 0.00936**

    - **# of Line Crossings:** 59, 72, 108, 187, 145, 88, 61, 46, 126, 17, 94, 145, 103, 93, 177, 128, 132, 141
      - **n = 18, M = 106.8 SD = 45.4**

  - **VPA**
    - **Time (s) in the Bottom Zone:** 233.5, 195.3, 197.1, 155.6, 170.4, 280.1, 280.4, 267.6, 215.1, 240.2, 219.4, 141.8, 181.9, 199.4
      - **n = 14, M = 212.7, SD = 44.0**

- **Distance Travelled (m):** 2.824, 3.579, 3.611, 2.672, 5.339, 4.285, 3.580, 6.530, 7.470, 5.631, 6.214, 7.344, 5.947, 5.659
  - **n = 14, M = 5.05, SD = 1.61**

- **Average Speed (m/s):** 0.009, 0.012, 0.012, 0.009, 0.018, 0.014, 0.012, 0.022, 0.025, 0.019, 0.021, 0.024, 0.020, 0.019
  - **n = 14, M = 0.01686, SD = 0.00543**

- **# of Line Crossings:** 75, 116, 117, 76, 125, 60, 54, 100, 134, 95, 122, 137, 102, 107
  - **n = 14, M = 101.4, SD = 26.5**

  o **Results**
  - **Time (s) in the Bottom Zone:** P value and statistical significance: The two-tailed P value equals 0.0472
    By conventional criteria, this difference is considered to be statistically **SIGNIFICANT. t = 2.0699, df = 30, standard error of difference = 16.440**

  - **Distance Travelled (m):** The two-tailed P value equals 0.6962 By conventional criteria, this difference is considered to be **not statistically significant**.

  - **Average Speed (m/s):** The two-tailed P value equals 0.6876. By conventional criteria, this difference is considered to be **not statistically significant**.

  - **# of Line Crossings:** The two-tailed P value equals 0.6983. By conventional criteria, this difference is considered to be **not statistically significant.**

  -
- **60-dpf Locomotion**
  o **Control**
  - **Time (s) in the bottom zone:** 126.1, 151.0, 71.3, 202.5, 145.6, 47.9, 119.2, 198.7, 122.5, 140.0, 1.1, 230.2
    - **n = 12, M = 129.7, SD = 65.9**

  - **Distance travelled (m):** 8.668, 11.669, 16.463, 13.005, 13.366, 12.692, 10.089, 11.640, 13.160, 15.728, 12.344, 6.997
    - **n = 12, M = 12.15, SD = 2.67**

  - **Average Speed (m/s):** 0.029, 0.039, 0.055, 0.043, 0.045, 0.042, 0.034, 0.039, 0.044, 0.052, 0.041, 0.023
    - **n = 12, M = 0.0405, SD = 0.009**

  - **# of Line Crossings:** 175, 208, 295, 254, 218, 197, 177, 233, 301, 327, 256, 131
    - **n = 12, M = 231, SD = 58.2**

  o **VPA**

- **Time (s) in the Bottom Zone (seconds):** 199.1, 150.4, 170.5, 168.3, 189.2, 177.3, 196.7, 86.8, 107.9, 199.3, 118.7, 101.8, 190.5, 156.3, 182.1
  - **n = 15, M = 159.7, SD = 38.2**

- **Distance Travelled (m):** 7.186, 11.257, 11.513, 8.666, 10.828, 15.649, 16.053, 15.171, 14.772, 10.549, 7.945, 8.232, 9.367, 7.831, 9.512
  - **n = 15, M = 10.97, SD = 3.06**

- **Average Speed (m/s):** 0.024, 0.038, 0.038, 0.029, 0.036, 0.052, 0.054, 0.051, 0.049, 0.035, 0.026, 0.027, 0.031, 0.026, 0.032
  - **n = 15, M = 0.0365, SD = 0.01033**

- **# of Line Crossings:** 169, 259, 232, 154, 230, 344, 266, 317, 305, 219, 195, 232, 250, 204, 243
  - **n = 15, M = 241.3, SD = 52.5**

- **Results:**
  - **Time (s) in the Bottom Zone**: The two-tailed P value equals 0.1508. By conventional criteria, this difference is considered to **be not statistically significant.**

  - **Distance Travelled (m):** The two-tailed P value equals 0.3014. By conventional criteria, this difference is considered to be **not statistically significant.**

  - **Average Speed (m/s):** The two-tailed P value equals 0.3044
    By conventional criteria, this difference is considered to be **not statistically significant.**

  - **# of Line Crossings**: The two-tailed P value equals 0.6344
    By conventional criteria, this difference is considered to be **not statistically significant.**

7-DPF TEST RESULTS AND STATISTICS

- **Control 1-1**: 11, 9, 9, 8, 6, 7, 5, 4, 2, 3, 3, 2
  - **n = 12, M = 5.75, SD = 3.05**

- **VPA 1-1**: 13, 8, 6, 4, 5, 4, 4, 4, 5, 5, 3, 4
  - **n = 12, M = 5.42, SD = 2.71**

- **Results:** The two-tailed P value equals 0.7799. By conventional criteria, this difference is considered to be **not statistically significant**.

SOCIAL PREFERENCE RESULTS AND SIGNIFICANCE

- **70-dpf Control**
    - **Time (s) in Zone 1:** 300.0, 138.1, 178.4, 218.9, 112.6, 300.0, 300.0, 98.6, 53.5, 162.8, 24.1, 298.9, 253.6, 113.6, 300.0, 299.0, 298.9, 300.0, 300.0
        - **N = 19, M = 213.2, SD = 98.0**

    - **Time (s) in Zone 2:** 1.1, 18.4, 53.9, 0.0, 1.0, 1.1, 0.0, 0.0, 0.0, 45.8, 39.3, 29.3, 48.5, 0.0, 0.0, 60.9, 40.1, 48.7, 23.9
        - **n = 19, M = 21.7, SD = 22.9**
    - **Combined Time (s) in Zones 1 and 2 (Social Half): n = 38, M =** 117.447368, SD = 119.758373

    - **Time (s) in Zone 3:** 0.0, 12.8, 58.4, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 50.0, 39.6, 37.2, 50.3, 0.0, 0.0, 53.1, 59.6, 40.4, 46.6
        - **n = 19, M = 23.6, SD = 24.9**

    - **Time (s) in Zone 4:** 0.0, 15.3, 74.2, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 66.1, 42.7, 14.6, 88.7, 0.0, 0.0, 87.5, 146.9, 48.1, 205.4
        - **n = 19, M = 41.5, SD = 58.2**
    - **Zones 3 and 4 Combined (Empty Zone): n = 38, M = 32.5657895, SD = 45.0775684**

    - **Total Distance Travelled (m):** 2.262, 5.697, 16.161, 1.743, 2.521, 2.337, 3.388, 1.760, 0.763, 8.679, 8.561, 7.044, 10.055, 0.000, 0.025, 11.769, 13.381, 12.001, 9.902
        - **n = 19, M = 6.21, SD = 5.02**

    - **Average Speed (m/s):** 0.008, 0.019, 0.054, 0.006, 0.008, 0.008, 0.011, 0.006, 0.003, 0.029, 0.029, 0.023, 0.034, 0.000, 0.000, 0.039, 0.045, 0.040, 0.033
        - **n = 19, M = 0.0208, SD = 0.0168 m/s**

    - **Number of Line Crossings:** 55, 54, 239, 37, 38, 65, 74, 9, 10, 166, 160, 88, 153, 0, 0, 229, 200, 236, 177
        - **n = 19, M = 104.7, SD = 85.3**

- **70-dpf VPA**
    - **Time (s) in Zone 1:** 101.1, 185.5, 169.9, 272.8, 184.6, 85.4, 264.7, 79.5, 208.0, 88.1, 95.0, 286.2, 228.2, 202.8, 254.1, 245.9
        - **n = 16, M = 184.5, SD= 73.6**

    - **Time (s) in Zone 2:** 78.0, 66.9, 59.2, 14.1, 40.8, 44.0, 11.4, 75.6, 22.7, 53.1, 86.8, 4.0, 27.4, 27.5, 23.4, 24.5
        - **n = 16, M = 41.2, SD = 25.9**

    - **Zones 1 and 2 Combined (Social Zone): n = 16, M = 112.85, SD = 90.8022985**

    - **Time (s) in Zone 3:** 63.9, 34.6, 35.7, 10.6, 39.3, 94.9, 15.9, 66.8, 23.9, 61.6, 76.6, 3.7, 21.1, 28.7, 12.4, 17.2
        - **n = 16, M = 37.9, SD = 26.9**

- o **Time (s) in Zone 4:** 63.9, 34.6, 35.7, 10.6, 39.3, 94.9, 15.9, 78.0, 45.4, 97.2, 41.5, 6.1, 23.3, 41.1, 10.2, 12.3
    - **n = 16, M = 40.6, SD = 29.3**

- o **Total Distance Travelled (m):** 9.742, 13.192, 9.366, 6.830, 9.728, 8.142, 4.564, 6.550, 7.670, 9.796, 9.220, 5.475, 5.452, 7.137, 3.480, 4.711
    - **N = 16, M = 7.57, SD = 2.54**

- o **Average Speed (m/s):** 0.032, 0.044, 0.031, 0.023, 0.032, 0.027, 0.015, 0.022, 0.026, 0.033, 0.031, 0.018, 0.018, 0.024, 0.012, 0.016
    - **n = 16, M = 0.025, SD = 0.0084**

- o **Number of Line Crossings:** 131, 186, 141, 139, 143, 134, 62, 116, 122, 168, 156, 77, 87, 131, 65, 55
    - **n = 16, M = 119.6, SD = 39.4**

- o **Results**
    - **Time (s) in Zone 1**: The two-tailed P value equals 0.3391. By conventional criteria, this difference is considered to be **not statistically significant.**

    - **Time (s) in Zone 4**: The two-tailed P value equals 0.9554. By conventional criteria, this difference is considered to be **not statistically significant.**

    - **Combined Time (s) in Zones 1 and 2 (Social half)**: The two-tailed P value equals 0.8591. By conventional criteria, this difference is considered to be **not statistically significant.**

    - **Total Distance Travelled (m)**: The two-tailed P value equals 0.3349. By conventional criteria, this difference is considered to be **not statistically significant.**

    - **Average Speed (m/s)**: The two-tailed P value equals 0.3707. By conventional criteria, this difference is considered to be **not statistically significant**.

    - **Number of Line Crossings**: The two-tailed P value equals 0.5262. By conventional criteria, this difference is considered to be **not statistically significant.**

II.

Validating Behavior in Zebrafish Models of Autism Spectrum Disorder Research

Mackenzie Moon - Weber State University

INTRODUCTION / PURPOSE

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication, repetitive behaviors, and impaired cognitive flexibility. There are many factors that cause autism, but one factor that is consistently observed is an alteration in the process that forms synapses (synaptogenesis). When certain genes are expressed incorrectly, the process of synaptogenesis may be disrupted, resulting in behavioral changes.

Our current research studies ASD in a zebrafish (Danio rerio) model in which embryos are treated at 0-48 hours post fertilization with valproic acid (VPA). This substance, known to induce autism-like symptoms in human children who were exposed in-utero, has also been known to cause these behaviors to manifest in zebrafish. Control groups consisting of untreated wild-type fish, and experimental groups consisting of fish treated with valproic acid will be put through a series of behavioral tests at different stages of development to validate a behavioral model of autism in zebrafish

PROBLEM

Expression of genes associated with autism is only one piece of the puzzle. Without validation of autism-like behaviors, we don't get the full picture autism spectrum disorder research.

LITERATURE REVIEW

Zimmerman et al. (2015) studied behaviors of ASD using zebrafish exposed to 48 μM Valproic Acid (VPA) for the first 48 of life. These VPA treated fish, as well as a control group were put through a series of tests at 6, 30, 70, and 120 dpf to evaluate locomotion, anxiety, aggression, and memory behaviors. The researchers hypothesized that VPA-treated fish would exhibit behaviors commonly associated with autism in humans.

The use of a zebrafish model in biomedical research provides a simpler, faster, and more cost-effective alternative to the traditional rodent model. Dwivedi et al. (2019) utilized a zebrafish model when studying the effects of VPA on abnormal behavior and gene expression. Experimental fish were exposed to 75 μM of VPA between 4 hours and 5 days post-fertilization. Behavioral tests were run between 7 and 21 dpf, and gene expression was measure using Real-time PCR.

References

Zimmerman, F. F., Gaspary, K. V., Leite, C. E., De Paula Cognato, G., & Bonan, C. D. (2015). Embryological exposure to valproic acid induces social interaction deficits in zebrafish (Danio rerio): A developmental behavior analysis. Neurotoxicology and Teratology, 52(PT A), 36-41. https://doi.org/10.1016/j.ntt.2015.10.002

Dwivedi, S., Medishetti, R., Rani, R., Sevilimedu, A., Kulkarni, P., & Yogeeswari, P. (2019). Larval zebrafish model for studying the effects of valproic acid on neurodevelopment: An approach towards modeling autism. Journal of Pharmacological and Toxicological Methods, 95, 56-65. https://doi.org/10.1016/j.vascn.2018.11.006

METHODS

Zebrafish Husbandry

Stock zebrafish were maintained in an aquatic habitat system with a constant temperature of 28 degrees Celsius. The habitat was kept in a room with a 14/10 hour light/dark cycle.

Adult, wildtype zebrafish were mated using mating tanks with dividers to separate male and female fish. At a scheduled time the dividers were pulled. Tanks were checked at 15-minute intervals and any embryos were immediately placed into petri dishes containing embryo medium. From there they were randomly separated into control and experimental groups.

These fish will be maintained in the same aquatic habitat system as the breeder fish.

Valproic Acid

Experimental groups were placed in 30 ml of embryo medium treated with 50 μM of Valproic Acid (VPA) for the first 48 hours after fertilization.

The VPA solution was changed after 24 hours. After 48 hours the embryos were placed in dishes containing only embryo medium.

Test Battery

- Thigmotaxis/locomotion performed at 6 dpf. Larvae were placed in a 24 well plate and observed for 5 minutes after a 1-minute acclimation period. Wall sticking behavior is indicative of anxiety.
- Open-field test at 30 dpf, 60 dpf to text anxiety in a novel environment. A greater amount of time spent in the lower half of the tank is indicative of anxiety.
- Mirror Response test at 60 and 120 dpf to test aggression.
- Social Preference test at 60 and 120 dpf to test social deficits and behaviors. Individual fish will be placed in a central tank with an empty tank on one side and a tank containing stimulus fish on the other. Time spent on either side indicates social preference.
- Circling behaviors will be assessed from recordings of locomotion and open-field tests.

Recordings of the tests to be analyzed using AnyMaze behavioral tracking software.
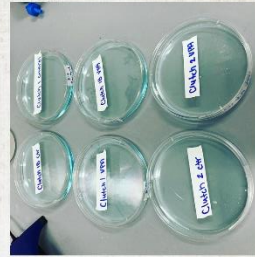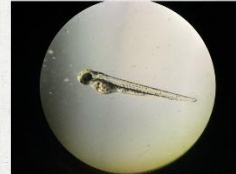
RESULTS

At the time of presentation only the 6-day post fertilization tests have been run and analyzed.

The average amount of time in seconds spent by the VPA group in the outer zone ($M = 252.53$, $SD = 36.008$) was greater than the amount of time spent by the control group ($M = 209.04$, $SD = 60.39$). This difference was statistically significant, $t = 2.1202$, $p < .05$

6 dpf Locomotion

Final results and analysis for this research are projected to be available at the end of the Summer 2021 semester.

ACKNOWLEDGEMENTS

Thank you to Dr. Elizabeth Sandquist and Dr. Jim Hutchins for their excellent mentorship and countless hours of assistance with this research.

Thank you to The Office of Undergraduate Research, the Weber State University Neuroscience department, and the Health Sciences department for the generous contributions to make this possible.

Mackenzie Moon
Weber State University
BIS Neuroscience, Psychology, Health Sciences
mackenziemoon@mail.weber.edu

WEBER STATE UNIVERSITY

III.

Long Term Grant Application
Cover Sheet

Amount Requested: $1994

Project Information

Moon, Mackenzie
Student Participant (Last, First)

Project Title: Validating Behaviors in Zebrafish Models of Autism Spectrum Disorder Research

_Hutchins, Jim_____3909_____
Faculty Mentor Name (last, first)   Mail Code

_Dumke College of Health Professions_____Health Sciences_____
College (Weber State is the University, NOT college)   Department

This project X DOES/__ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

_____   _____
Student Signature   Date

Attach Mentor Email
Must be 10 business days before final deadline.   ___3909_____   ____x6505_____
                                                                        Campus Mail   Phone Ext.

Attach Research Committee Representative Email
Date Received by URC Rep. must be 5 business days before final deadline.

Attach Mentor Department Chair Email
Faculty Mentor Department Chair

Please check if attended Research Proposal Workshop:

| X |

Date Workshop attended/PPT Requested: 08/19/2020

x

Student Information Form

Project Title

Validating Behaviors in Zebrafish Models of Autism Spectrum Disorder Research

_____

____

Student Information

Moon, Mackenzie	W01349626
_____	
Student Name (last, first)	Student ID#
(801) 300-5719	mackenziemoon@mail.weber.edu
_____	
_____	
Phone	Weber Email
107	Summer 2021
_____	
Total Number of Credits Completed	Anticipated Graduation (term/year*) (*funds may NOT be spent after graduation)

_____   _____

Student Signature   Date

Please make additional copies of this form for additional students.

Budget Worksheet

BUDGET ITEM	Department or College Funds	Outside Agency Funds	Personal Funds	Undergrad. Research Funds	GRAND TOTAL
Materials	Neuroscience: $500 for materials			• 3 6-well plates @ 100.93=302.79 • 1 24-well plate 153.62 • Petri dishes 94.40 • 1 Polycarbonate Lexan clear plastic sheet 43.99 • 3 Polycarbonate clear plastic sheets @ 25.99=77.97 • Aquarium sealant 9.39 • Black nail color 1.97 • 1 agarose gel 179.78 • 1 PTCH2sa fish line 450.00 • 1 la016356Tg fish line 450.00 • Fish line shipping 2@150.00=300.00 Total: $2064-$500=$1564	$2064
Equipment	Dept. of Health Sciences (in-kind ARCC donation): $500 Neuroscience: video camera (in kind donation) $350 Jim Hutchins (mentor, in kind donation) Desktop Computer $100 Dept. of Zoology (in kind donation for husbandry) $490	ARCC: $5995 toward ANYMaze software		10.2" iPad $430	$7865
Research Scholarship (max request $2,500.00)					
Mileage to gather Data (.38 per mile)					

GRAND TOTAL	$1940	$5995		$1994	$9929

Body of Proposal

Project Description

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication, repetitive behaviors (also known as "stimming"), and impaired cognitive flexibility. The symptoms of ASD typically manifest in children during the early developmental period, but in many cases, diagnosis may not occur until the child has entered the education system. Earlier detection and intervention lead to a better outcome for those on the autism spectrum (Fernell *et al*., 2013). The cause of autism is multifactorial, but one factor that is consistently observed is an alteration in the process of synaptogenesis. Synaptogenesis is the creation of synapses between neurons. This process is dependent on the expression of certain genes. When these genes are incorrectly expressed the process of synaptogenesis may be disrupted, causing behavioral deficits to manifest (Kozol, 2018).

Current research on the relationship of these genes in a zebrafish model of autism is being performed at Weber State University by Brittney Child under the direction of Dr. Jim Hutchins and Dr. Elizabeth Sandquist. The genes under investigation include PTCH2 and lmx1bb. This research involves the use of zebrafish (*Danio rerio*), which are commonly used in ASD research because of their rapid life cycle, transparent embryos that allow for easier observation of development, and the relative ease of creating genetic mutants for testing. The proposed research involves exposing zebrafish embryos to valproic acid (VPA) to induce autistic characteristics and behaviors. VPA is also known to alter expression of genes involved in autism (Baronio *et al*.,

2017). The altered expression of ASD-related genes on its own does not show the whole picture. The model must be tested behaviorally to tie the behavioral deficits to the gene being tested. Though these are separate projects, Ms. Child and I will be correlating our results. The genetic and behavioral aspects of autism research each have their own pieces that together allow a greater understanding of the puzzle of autism.

I have chosen Dr. Jim Hutchins as my mentor for this project because of his knowledge and experience in the fields of developmental neuroscience and genetics. Dr. Hutchins will be advising me as I navigate the process of this research, but I will be performing the experiments and analyzing the data.

    Dependent _____X_____ Independent

    (student helping faculty do research)   (student doing own research)

    I am the mother of three children on the autism spectrum. As such, I have gained a unique perspective of the behaviors that accompany autism spectrum disorder and a heightened desire to understand the science behind it. I am currently a senior at Weber State University majoring in psychology and minoring in neuroscience. I am in the process of transitioning to a BIS in neuroscience, psychology, and health sciences. I have taken courses in introductory neuroscience, cellular and molecular neuroscience, clinical neuroscience, biology, anatomy and physiology, and psychology that have helped prepare me for undergraduate research. I have been trained in zebrafish husbandry by Dr. Sandquist and have completed IACUC training to prepare for working in the lab.

Project Methods & Timeline

Wild-type zebrafish will be mated, and the resulting embryos will be separated into two groups: Experimental fish treated with valproic acid, and untreated control fish. The experimental fish will be exposed to 50 µM of VPA at 0-48 hours post-fertilization to induce the behaviors and characteristics of autism. We will also test zebrafish whose genetics are altered in the same location as the genes Ms. Child is studying (the PTCH2sa & la016356Tg mutant/transgenic lines for PTCH2 and lmx1bb, respectively). The fish will undergo behavioral tests at 30-, 70-, and 120-days post-fertilization (dpf).

Tests to be performed will analyze locomotion, social behaviors, aggression, anxiety, and inattentive behavior. Locomotion and anxiety will be measured with an open-field test that involves placing the fish in a tank with marked sections. Time spent in each section indicates levels of anxiety. Social interaction will be measured using mirror stimulation response. A second test to measure social interaction, a social preference test, will place the experimental fish in a tank with an identical tank on either side. One tank will be vacant while the other contains stimulus fish. Time spent near either tank is indicative of social interaction and anxiety (Zimmerman *et al.*, 2015). Repetitive behaviors ("stimming") will be tested by recording the circling behaviors of the fish. Inattentive behaviors will be measured by introducing the fish to an aversive stimulus. The fish will be placed in a clear plastic tray which will be positioned over a tablet screen displaying a red line (Dwivedi *et al.*, 2019). This digital red line is the aversive stimulus. A timeline for this project is outlined in the table below:

January	February	March	April

• Assembling tanks • Mating fish for VPA treatment • Obtain mutant/transgenic lines • Trial runs	• Open field test • Inattentive behavior test • Circling behavior test • Social preference test • Husbandry • Data analysis • 2021 UCUR presentation	• Open field test • Mirror response test • Husbandry • Data analysis • OUR Symposium Presentation	• Preparation for 120-dpf testing • Husbandry • Data analysis
May	**June**	**July**	**August**
• Mirror response test • Social preference test • Open field fest • Husbandry • Data analysis	• Open field test • Inattentive behavior test • Circling behavior test • Social preference test • Husbandry • Data analysis	• Open field test • Inattentive behavior test • Circling behavior test • Social preference test • Husbandry • Data analysis	• Conclusion of research • Data analysis • Poster creation • Written paper detailing research • Preparation for 2021 Society for Neuroscience meeting

The behavioral tests will be recorded with a camera and analyzed using the ANY-Maze software.

The methodology, and any available preliminary data, will be presented at the 2021 UCUR and

2021

Undergraduate Research Symposium. A complete set of data from this project will be available by

August 2021. Promising results will be used for future presentations at meetings such as the 2021

Society for Neuroscience meeting, continuing research, and submission of a scientific paper for

possible publication in a journal such as *The Journal of Autism and Developmental Disorders.*

Budget Explanation

Materials and equipment: Dr. Sandquist has graciously allowed us to use her lab, including her fish colonies, for our experiments. The necessary ANY-Maze software and video behavioral tracking equipment is being provided by in-kind contributions from the ARCC, the Neuroscience Program, and Dr. Hutchins. Consumable supplies are listed in the table below.

Item	Vendor	Cat Number	Quan	Cost
Falcon™ Polystyrene Microplates: 6-well plate (50/cs)	Fisher	08-772-49	3	302.79
Falcon™ Polystyrene Microplates: 24-well plate	Fisher	08-772-51	1	153.62
Falcon™ Polystyrene Petri Dishes	Fisher		1	94.40
Polycarbonate Lexan Clear Plastic Sheet 1/8" x 24" x 48"	Amazon		1	43.99
Polycarbonate Lexan Clear Plastic Sheet 1/16" x 12" x 24"	Amazon		3	77.97
All-Glass Clear Silicone Aquarium Sealant, 3 oz.	Petco		1	9.39
Sally Hansen Hard as Nails Color, Black Heart, 0.45 fl oz	WalMart		1	1.97
Agarose (Low-EEO Multi-Purpose Mol Biol Grade)	Fisher	BP160-500	1	179.78
ptch2sa19807 Fish Line	Zfin	ZL10948.01	1	450.00
la016356Tg Fish Line	Zfin	ZL8596.07	1	450.00
Fish line shipping	Zfin		2	300.00

The total budget for this project is $9929. In-kind donations of $1940 were obtained from the

Neuroscience Program, Dept. of Health Sciences, Dept. of Zoology, and Dr. Jim Hutchins.

ANY-Maze software purchase was offset by a grant of $5995 from the ARCC/Dee Family

Foundation to Dr. Jim

Hutchins. The amount requested from the Office of Undergraduate Research is $1994.

Long Term Grant Application
Additional Questions

1. What funding have you received from OUR in the past? Where has your previous project been disseminated?

   I have not received any previous funding from the OUR.

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department.

This project is not part of a required course, but I will be receiving credit through NEUR 4800 Research and Projects.

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining?

Additional sources of funding have been received in the amounts of $5995 from the Academic Resources and Computing Committee (ARCC) Dee Family Grant

$500 from the Dept of Health Sciences. A contribution from the Neuroscience Program in the amount of $500 has also been solicited.

4. Where do you plan to disseminate the results of this project?

I plan to share my preliminary findings at the 2021 WSU OUR Symposium. This preliminary data will also be presented at local spring 2021 conferences, and at a national conference if feasible. Promising results will be used for future presentations, continuing research, and possible publication.

5. If you are requesting a Research Scholarship, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules.

I am not requesting a research scholarship at this time.

Appendix 1: References

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Kozol, R. A. (2018). Prenatal neuropathologies in autism spectrum disorder and

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Baronio, D., Puttonen, H. A. J., Sundvik, M., Semenova, S., Lehtonen, E., & Panula, P.

  (2018). Embryonic exposure to valproic acid affects the histaminergic system and

  the social behaviour of adult zebrafish (danio rerio). *British Journal of*

  Pharmacology, 175(5), 797809. doi:10.1111/bph.14124

Zimmermann, F. F., Gaspary, K. V., Leite, C. E., De Paula Cognato, G., & Bonan, C. D.
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Embryological exposure to valproic acid induces social interaction deficits in zebrafish

  (danio rerio): A developmental behavior analysis. *Neurotoxicology and*

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  (2019). Larval zebrafish model for studying the effects of valproic acid on

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  Pharmacological and Toxicological Methods, 95, 56-65.

  doi:10.1016/j.vascn.2018.11.006

LONG TERM GRANT APPLICATION
Faculty Recommendation Form

Student Name (last, first): Moon, Mackenzie

Project Title: Validating Behaviors in Zebrafish Models of Autism Spectrum Disorder Research

Mentor Directions: After carefully reviewing the proposal and assessing both the viability of this project and the qualifications of the student requesting funding, answer the questions found below. Please expand the sections as necessary (**do not attach separate letter**). If the project involves the use of human subjects or protected animals, be sure the student secures IRB or ACUC approval. If the project receives funding, it is your responsibility to work closely with the student, monitor the ongoing progress of the project and budget, and evaluate the project's results. Failure to do so will jeopardize funding for this project and any future projects.

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1. How long and in what capacity have you known this student?

1 ½ years: I have known Mackenzie ("Macy") since she was my Intro Neuroscience student in summer 2019. During fall 2019 and spring 2020 we began discussing this research project in weekly lab meetings.

2. Briefly describe the proposed project. Is this part of a larger research project? Is this part of a course? If so, how is the project apart from the nature and scope of activities normally taken for the course (Please attach a copy of your course syllabus)?

The project is to assess autistic-like behaviors in a zebrafish model of human autism. We know that most, if not all, autistic spectrum disorders stem from a defect in normal synaptogenesis. (A synapse is a contact between two nerve cells; there are 1,000,000,000,000,000 of these contacts in the human brain. Synaptogenesis is the process that forms these contacts.) It's obvious that's too complex a problem to work out without some simplification; we propose to use the well-established zebrafish model of human autism to study the foundational mechanisms of human autism. Brittney Child, an OUR grantee, is studying the levels of six different genes (including PTCH2 and lmx1bb) to see if they change in a drug (valproic acid)-treated model of autism. There are also mutants for these genes. We propose to study whether there are behaviors in valproic acid-treated zebrafish, or mutant zebrafish, which mimic human autism.

3. Give an assessment of the project's significance to the student's discipline and of the project's educational and/or professional benefit to the student.

Macy obviously has an interest in autism as she is the mother of autistic children. She combines this with a passion for research. She plans to attend graduate school in the neurosciences. Successful completion of this project will help her understand the principles of biomedical research and serve as a springboard that gets her into a great research lab for her PhD dissertation research.

4.   Comment on the qualifications of the student to successfully complete this project, both in terms of the project's scope and its time frame.

My time, like that of most Weber State faculty, is quite limited. I need a student who is a brilliant selfstarter. Macy is that student. She has presented the peer-reviewed literature to me and designed her own research project. I am confident that she has a better-than-average chance that her research will result in a publication. Dr. Sandquist and I want to see our students' research disseminated and for our students to derive the educational and professional benefits that a research publication will bring. Presentation at UCUR and the Spring Symposium is a necessary but not sufficient condition for a student-driven research publication with a payoff by her graduation of August 2021.

5.   Comment on the justification and appropriateness of the project budget, including the necessity of a Research Scholarship (if requesting one).

I have carefully reviewed the budget and find it sufficient for this project. We have already invested a substantial amount of money in the camera, computer, and software for tracking behavior (see in-kind contributions on the budget). Now it's up to Macy to leverage that with a smaller amount of additional funding specific to her project.

6. Describe your role in the project.

Dr. Sandquist and I feel that students benefit most when they are given the independence to complete a project on their own. I have supervised Macy closely at each step but my role is more in suggesting possible avenues for her research, reviewing the research data to ensure it was properly gathered, and helping her properly disseminate her research data once it's gathered.

7. Include anything else that you think will be helpful to the committee in evaluating this application.

I want the committee to know I think the world of both Brittney and Macy. Brittney is already well on her way to a research publication from the OUR-funded work she has done this summer. Macy is equally capable and I'm sanguine that between these two students our lab will develop work suitable for publication.

This project ___X_ DOES ____ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Dr. Jim Hutchins is PI on an existing IACUC protocol, 20-01, under which this research is covered.

In place of your signature, please email this form as an attachment for the student to add to the final proposal.

3909   x6505
Campus Mail Code   Phone Extension

IV.

BIS CAPSTONE PROPOSAL

Thesis Question: Do zebrafish treated with valproic acid display autistic behaviors analogous to humans with autism spectrum disorder? How do the behaviors of the test group differ from those of the untreated control group?

What I intend to do: I intend to mate wildtype zebrafish and expose the experimental group to Valproic acid between 0- and 48-hours post-fertilization. Starting at 6 days post-fertilization I will begin a series of behavior tests with both groups to determine the appearance of autism-like behaviors, as well as measure the difference in behaviors between the treated and untreated fish. My capstone project will be a combination of a 10-to-15-page primary research paper and a PowerPoint presentation detailing the research I am conducting. The paper will also contain a review of the literature used to prepare for this research. The project will bring together knowledge from each of my areas of emphasis: Neuroscience, psychology, and health sciences.

Why I intend to do it: As a mother of three autistic children, I have gained a unique perspective of autistic behaviors. I wish to further my understanding of the physiological and behavioral aspects of autism spectrum disorder through research. This research will also give me valuable experience in preparation for a doctoral program. On a larger scale, deeper understanding of ASD could allow researchers to develop more effective treatments for the symptoms of autism.

My step-by-step plan: I am fortunate to have begun this research in January of 2020. Much of my step-by-step plan has been accomplished at the time that this paper is being written. I will outline the process I have completed to this point and the steps to be completed.

Step 1: Familiarize myself with the zebrafish model of autism through a review of peer-reviewed literature. I began this process with directed readings with Dr. Jim Hutchins during the 2020 spring semester. This process continued into the 2020 summer semester with directed readings and journal club with Dr. Hutchins and Dr. Sandquist. As a group we reviewed articles on zebrafish research, genetic variations in zebrafish, and genes that contribute to autism spectrum disorder. Further research is needed into the neuroanatomy and neurological processes in humans.

Step 2: Further familiarize myself with autism spectrum disorder and behavioral treatments. Expand my research of autism spectrum disorder by finding 3 to 5 peer-reviewed articles for each area of emphasis.

Step 3: Complete lab training and certifications needed to participate in lab activities such as animal husbandry. I completed my training and have been working in Dr. Sandquist's zebrafish lab since fall 2020.

Step 4: Write and submit an OUR grant proposal to obtain funding for my research project. My grant proposal was submitted in October 2020 and received full funding.

Step 5: Complete planned timeline for behavioral tests.

Test	ASD trait	When	Author
Open-Field	Thigmotaxis/Anxiety	6-dpf, 30-dpf, 60-dpf	Zimmerman
Inattentive Behavior	Cognitive flexibility, habituation	7-dpf, 21-dpf	Dwivedi
Circling	Repetitive behaviors	7-dpf, 21-dpf	Dwivedi
Social Preference test	Social deficits	70-dpf	Zimmerman
Mirror-Response	Social deficits, aggression	75-dpf to allow fish to recover from 70-dpf testing	Zimmerman

*Note: Circling and inattentive behavior tests will be performed on most recent yield of embryos at 7- and 21-dpf and analyzed by hand. I will require assistance with the agarose to create the lanes for the inattentive behavior test.

Step 6: Mate wildtype zebrafish to collect embryos for tests. We successfully mated the fish and collected embryos for testing at the beginning of March. Embryos were collected immediately after fertilization and separated into two groups: Control and experimental. Experimental fish were placed in a container of 30 mL of embryo medium treated with 50 μM of valproic acid (VPA) for the first 48-hour post-fertilization. Control fish were maintained in 30 mL of untreated embryo medium.

Step 7: Conduct tests at 6, 30, 60, 70 days post-fertilization.

Step 8: Analyze tests using ANYmaze behavior tracking software.

Step 9: Write up results and their meaning.

Step 10: Incorporate results into final paper and presentation.

Step 11: Present research in person to committee, as well as students and faculty from related departments at a scheduled lecture.

Final Product: The final product will be a 10-to-15-page research paper and PowerPoint presentation containing details of my research project, an overview of autism spectrum disorder, and a literature review.

REFERENCES

Dwivedi, S., Medishetti, R., Rani, R., Sevilimedu, A., Kulkarni, P., & Yogeeswari, P. (2019).

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V.

ANY-maze

Stoelting Co.
620 Wheat Lane
Wood Dale, IL 60191, USA

Jim Hutchins, Ph.D.
Weber State University
jimhutchins@weber.edu

July 23, 2021

Dear Jim Hutchins, Ph.D.,

Thank you for your recent purchase (WEB244341) of the ANY-maze Video Tracking System software.

Based on your serial number: 5GTT-8HNV-UFXA-SRT5, your ANY-maze license number is:

MUCX-JDWC-J8XR-AZWA

Lifetime technical support and upgrades are included with the purchase of each ANY-maze license.

To activate your license follow these steps:

1. If the copy of ANY-maze you want to license is running on THIS machine, then it's a good idea to copy the license number to the clipboard now.
2. Start ANY-maze and switch to the *Support* page, by clicking the *Support* tab at the top-right of the window.
3. On the Support page, choose the *Licensing* option, shown on the left side of the page. The *ANY-maze License Manager* will open.
4. In the ribbon bar, select the option to *Activate a license*.
5. If you copied the license number to the clipboard then it will already be shown on this page, otherwise type the license number into the four boxes shown.
6. Finally, click the *Activate now* button. A page will be shown informing you that the license has been activated successfully.

If you have any questions about your order, please contact: orders@stoeltingco.com, or, if you have any technical support questions, please contact: techsupport@anymaze.com. Alternatively you can contact us by phone on our toll free number 800-860-9775, or if you are outside the USA, + 1 630-860-9700.

Once again, thank you for your purchase and we hope we can be of service to you in the future.

Sincerely,

The ANY-maze Team