MATERNAL T CELL IMMUNE ACTIVATION AND POSTNATAL STRESS: A TWO HIT MOUSE MODEL OF SCHIZOPHRENIA

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ABSTRACT OF THE THESIS

Maternal T Cell Immune Activation and Postnatal Stress:

A Two Hit Mouse Model of Schizophrenia

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Prenatal maternal immune activation (MIA) is a risk factor for several psychopathologic disorders, including bipolar disorder, autism, and schizophrenia. Most research has focused on using toll-like receptor agonists such as lipopolysaccharide or the synthetic viral genome analogue polyinosinic:polycytidylic acid as experimental immune activators. Little is known about how T cells, necessary for successful clearance of viruses and bacteria, may influence the neurodevelopment of the gestating fetus. Additionally, MIA has been implicated as the first hit in a two-hit hypothesis of schizophrenia development when coupled with a secondary insult, such as psychological stress, during adolescent neurodevelopment.

To this end, I challenged pregnant C57BL/6J mice on day E12.5 of gestation with 200ug/kg of purified staphylococcal enterotoxin A (SEA), a potent superantigen, to oligoclonally activate maternal T cells. After birth, both male and female offspring were randomized into “stress” or “no stress” groups, where “stress” animals would experience a 14 day battery of mild, unpredictable stressors. All animals were then tested for spatial navigation in a water radial arm maze (wRAM), anxiety-like behavior in the elevated plus maze (EPM) and open-field, and sensorimotor gating, as measured by prepulse inhibition (PPI).

In the wRAM, during hidden platform training, SEA-No Stress animals took significantly longer to find the escape platform and traveled significantly farther before finding it. SEA-No Stress animals also had a significantly higher number of fail errors, where they did not find the platform within 60 seconds. When testing for spatial memory, SEA-Stress animals spent the least amount of time in the platform arm, as well as taking significantly longer to make first entry. Stressed animals traveled further total distance in both the EPM and open field. Animals from SEA treated mothers spent less time investigating a novel object in the open field, as well as spent more time on the perimeter of the maze. No differences were observed between animals in PPI.

Together, these findings suggest that specific T cell activation during pregnancy alters behavioral development in the offspring. Additionally, adolescent stress does not have an additive effect on prenatal SEA treatment, but rather combines to form a unique phenotype.

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Table of contents

Abstract	pg. ii
Acknowledgements	pg. iv
List of tables & figures	pg. vi
Overview of Thesis	pg. 1
Introduction	pg. 2
Specific Aims	pg. 9
Research Design and Methods	pg. 11
Results	pg. 20
Discussion	pg. 27
Tables and Figures	pg. 32
References	pg. 54

List of tables & figures

Table 1: Behavioral test schedule for all animals pg. 30

Table 2: Randomized stressor schedule pg. 31

Figure 1: Animal body weight on day 0 of stress, faceted by sex pg. 32

Figure 2: Animal body weight on day 15 of stress, faceted by sex pg. 33

Figure 3a: Grams of food eaten per animal per cage per day pg. 34

Figure 3b: Change in average food consumption over stress protocol pg. 35

Figure 4: Duration of time spent in the water radial arm maze pg. 36

Figure 5: Latency to find the hidden platform pg. 37

Figure 6: Number of fail errors made in the water radial arm maze pg. 38

Figure 7: Distance traveled in the wRAM pg. 39

Figure 8: Swim speed in the water radial arm maze pg. 40

Figure 9: Time spent in the target (platform) arm during probe trial pg. 41

Figure 10: Time to enter the target (platform) arm, faceted by sex pg. 42

Figure 11: Distance traveled in the elevated plus maze pg. 43

Figure 12: Number of entries into open arms in the elevated plus maze pg. 44

Figure 13: Distance traveled in the open field without object pg. 45

Figure 14: Time spent in the center of the open field without object pg. 46

Figure 15: Distance traveled in the open field with a novel object pg. 47

Figure 16: Time spent in the center of the open field with object pg. 48

Figure 17: Distance traveled in open field with and without object pg. 49

Figure 18: Time spent in the center area of the open field with object pg. 50

Figure 19: Average percent prepulse inhibition pg. 51

A. Overview of Thesis

Schizophrenia is a chronic, debilitating disease of the mind, thought to affect nearly one percent of the American population. Symptoms of schizophrenia include positive symptoms such as hallucinations (perceiving things that do not exist) and delusions (false beliefs such as believing they are somebody else, or that an external force is trying to control or kill them), as well as negative symptoms, such as flat affect (loss of emotionality in movement and speech) and anhedonia (loss of pleasure). Above and beyond the high toll this disease takes on the individual, schizophrenia places a heavy burden on the families of those affected and society. The estimated cost of schizophrenia in the United States in 2002 was 62.7 billion dollars, a large portion of which being due to high unemployment among people with the disease and the increased financial burden on support givers (Tajima-Pozo, de Castro Oller, Lewczuk, & Montanes-Rada, 2015). Better understanding of how this disease develops is critical for the development of effective interventions that increase the quality of life of those affected, and decrease the financial strain on our healthcare system.

One of the current theories on the development of schizophrenia is the two-hit hypothesis, which states that at least two genetic or environmental insults are necessary to precipitate schizophrenic symptoms (Feigenson, Kusnecov, & Silverstein, 2014). According to the hypothesis, the specific insult may vary, but must take place during a "sensitive period" of central nervous system development, such as primary fetal neurodevelopment or during adolescent neurodevelopment. The present thesis will focus on this line of research, testing the hypothesis that an insult during fetal neurodevelopment (maternal immune activation), coupled with an insult during adolescent neurodevelopment (psychological stress), will cause the development of schizophrenia-associated behaviors in an animal model.

B. Introduction

Schizophrenia is a complex disease that lacks a singular genetic or environmental pathogenic agent. Epidemiological studies have suggested that maternal illness during pregnancy may serve as a risk factor for the eventual development of schizophrenia in offspring (Jaaskelainen et al., 2015; Mednick, Machon, Huttunen, & Bonett, 1988). These studies have suggested a mechanism for the observed seasonality effect in schizophrenia diagnoses; in the northern hemisphere, people born in February or March have a higher risk to develop the disease compared to those born in other parts of the year. These offspring have gestated through the influenza season, which peaks in the final weeks of the calendar year (Appiah et al., 2015). Recent findings suggest that, rather than becoming directly infected, gestating offspring are exposed to the mother's activated immune system, which is working to clear the mother's infection (Hsiao & Patterson, 2012).

The Immune System

We are constantly exposed to foreign particles that are inhaled, ingested, sitting on our skin, or are stuck within our mucous membranes. Our defense against these particles, and the reason we do not always get sick, is our immune system (Muller, Weidinger, Leitner, & Schwarz, 2015). The immune system is comprised of cells, tissues, and organs that deciphers between what belongs and what does not belong in the body, and works to remove that which does not belong.

The immune system is split into two different branches: the innate system and the adaptive system (Kaufman et al., 2015). The innate system is comprised of cells that can broadly identify foreign particles. These particles could be virus, bacteria, parasites, or even a transplated organ. What the innate system lacks in specificity, it makes up for

in speed. Innate immune cells distributed throughout the body can start an immune response to a foreign particle minutes after it is introduced. On the other hand, the adaptive system is slow and specific. These cells require time to activate, but are specific to the individual particle. This is important because the specific response is required for complete removal of the foreign particle.

Nearly all immune cells are derived from hematopoietic stem cells of the bone marrow (Kumar & Jack, 2006). Hematopoietic stem cells differentiate into different types of cells: the common myloid progenitor cell and the common lymphoid progenitor cell (Eaves, 2015). The myloid progenitor cell differentiates into neutrophils, macrophages, and mast cells. Nearly all myloid cells are members of the innate branch of the immune system. These cells are important for the general identification of "self" versus "non-self", as well as the first response at the site of infection and general cleanup of cellular debris. Lymphoid progenitor cells differentiate into T cells and B cells. These cells are antigen-specific, meaning they only respond to a very specific portion of a specific invading particle. They make up the adaptive branch of the immune system and are necessary for the complete removal of the foreign particle from the body. Failure to recruit T and B cells during an infection can lead to the failure of particle clearance and either chronic disease, further complications, or even death (Sun & Braciale, 2013). Dendritic cells, which can be made by either myloid or lymphoid progenitor cells, function as the link between the innate and adaptive branches, providing the information required to activate adaptive cells via antigen presentation.

T Cell Immunity and Antigen Presentation

T cells are lymphoid cells that are part of the adaptive immune system. Unlike B cells, that are both formed and mature in the bone marrow, T cells form in the bone marrow and then migrate to the thymus to complete maturation (Xu et al., 2013). In the

thymus, T cells assemble a T cell receptor (TCR) that is able to recognize a specific antigen from a foreign particle (Jameson & Bevan, 1998). Mature T cells that have developed a proper TCR are released into the body, where they wait in lymph nodes for activation by dendritic cells.

Over the normal course of infection, dendritic cells capture debris from foreign particles through a process called phagocytosis. Once this happens, the dendritic cells go through a round of maturation, where they upregulate expression of a protein called the major histocompatibility complex (MHC) (Mellman & Steinman, 2001). This protein is able to hold the captured foreign debris on the cell surface of the dendritic cell. The dendritic cell then travels to the nearest lymph node, where it displays its foreign debris on the MHC protein to the waiting T cells. This process is known as antigen presentation (Adiko, Babdor, Gutierrez-Martinez, Guermonprez, & Saveanu, 2015). The dendritic cell presents this foreign material to the T cells until one of them activates, starting the adaptive immune response.

In order for the T cell to activate, the TCR needs to identify the foreign particle being displayed on the MHC of a passing dendritic cell. This happens by the CDR3 region of the TCR matching the topographic structure of the combined MHC-particle surface on the dendritic cell. While each T cell's specificity is conferred by the CDR3 region of their TCR, many TCRs share similar structural components. These are called V-alphas and V betas. TCRs may share the same V-beta component, but will be specific to different foreign particles based on their CDR3 region.

Bacterial Superantigens: Mechanism of Action

Over the course of an infection, T cells are activated through their TCR by dendritic cells presenting recognizable antigen on their surface MHC. To experimentally

study the role of T cells on the central nervous system (CNS), previous studies have used bacterial superantigens (SAgs) (Kusnecov, Liang, & Shurin, 1999). Superantigens are produced by bacteria (such as *S. aureus)* and drive an exaggerated T cell response by cross-linking the TCR and MHC molecules together. This drives T cell activation independent of the antigen being presented by the dendritic cell. The specificity of superantigens relies on the V-beta component of the T cell receptor. Since many T cells share these structural components, all will be activated by SAg activity. Because of this, SAgs engage a considerably higher number of unique T cells compared to antigen-specific activation alone (Proft & Fraser, 2003).

Maternal Immune Activation (MIA) and Deleterious Behavioral Outcomes in Offspring

The observation of seasonality in the diagnosis of schizophrenia (Mortensen et al., 1999), coupled with the increase in diagnosis after major viral epidemics (Mednick et al., 1988), led to the rise of work on the role of the maternal immune system in generating behavioral deficits in offspring. These studies activate the maternal immune system during pregnancy, then test the offspring for changes in behavior that may be indicative of abnormalities in neurodevelopment.

As schizophrenia is associated with deficits in the hippocampus (Gur & Gur, 2013), many of these studies focus on spatial learning tests for offspring of immune-activated mothers. Zhang and van Praag used Poly I:C on gestational day 15 to experimentally activate the macrophages of the maternal immune system and tested the offspring of these pregnancies at three months of age (Zhang & van Praag, 2015). These offspring exhibited decreased sensorimotor gating and worse spatial navigation in the morris water maze (measured by latency to find the hidden platform) and the aquatic T-maze (measured by correct arm alterations). Additionally, Zhang and van Praag found decreased neurogenesis in the dentate gyrus of offspring born to immune-activated

mothers, suggesting insult during *in utero* development may lead to long-term structural changes in the offspring hippocampus.

Makinodan et al. also used Poly I:C to activate the immune system of pregnant mice and found their offspring to have significantly less myelin basic protein in the hippocampus compared to saline treated controls (Makinodan et al., 2008). This difference only existed in early postnatal offspring, with these abnormalities returning to normal by adulthood. Behaviorally, these animals also exhibited decreased sensorimotor gating measured by prepulse inhibition. Additionally, maternal Poly I:C treatment has been shown to impair synaptic development of upper layer neurons in the cerebral cortex (Soumiya, Fukumitsu, & Furukawa, 2011).

Anxiety- and depression-like behaviors have also been associated with maternal immune activation (Enayati et al., 2012). Babri, Doosti, and Salari tested both NMRI and C57BL/6 animals in a MIA study that used lipopolyscharride (LPS) as the immunostimulant (Babri, Doosti, & Salari, 2014). LPS, like Poly I:C, activates macrophages. They found offspring from immune-activated mothers had increased anxiety-like behavior in the elevated plus maze, but only for NMRI animals. C57BL/6 offspring did not show an increase in either anxiety- or depression-like behaviors. Their findings suggest that maternal immune activation may alter emotional behavior, but in a strain-dependent manner.

The "Two Hit" Model of Schizophrenia Development

To try and explain the existence of schizophrenia despite the lack of a single causal agent, research has shifted to a "two-hit" model of disease onset (Maynard, Sikich, Lieberman, & LaMantia, 2001). Two-hit models of schizophrenia development are similar to other two-hit models proposed for other complex diseases, such as cancer.

These hypotheses state that a prenatal genetic disposition or environmental insult acts as a “first hit” that then sensitizes the subject’s nervous system to an environmental “second hit” later in life (Feigenson et al., 2014). The combination of the downstream effects of these two hits then precipitates disease onset. Either hit alone is not enough to cause an observable disease phenotype.

Much research has been done on the development of behaviors associated with schizophrenia in animal models, using different types of genetic or environmental insults as either the first or second hit in the two-hit model. Klug and van den Buuse (2013) investigated how genetic susceptibility could act as a first hit by using animals with a mutation in the gene that encodes brain derived neurotrophic factor (BDNF). These animals had roughly half the amount of BDNF during primary neurodevelopment (Klug & van den Buuse, 2013). These animals did not have an overt schizophrenic phenotype, though if they were exposed to chronic cannabinoid receptor stimulation during adolescence, they would develop deficits in sensorimotor gating and would show increased neophobia. Only the animals exposed to both low BDNF (BDNF +/- genotype) and adolescent cannabinoid receptor stimulation showed deficits analogous to behaviors seen in humans with schizophrenia.

Genetic sensitivity is not the only type of first hit that can sensitize offspring to secondary environmental hits later in life. Based on the epidemiological evidence in humans (Mednick et al., 1988; Mortensen et al., 1999) and work in the field of maternal immune activation, researchers have started to investigate the role of the maternal immune system as a potential environmental “first hit” in the two-hit model of schizophrenia development (Feigenson et al., 2014; Meyer et al., 2008). Walker et al. (2010) found that maternal exposure to LPS could contribute to the development of schizophrenia-associated behaviors when paired with psychological stress in

adolescence (Walker, Nakamura, & Hodgson, 2010). Animals that received only adolescent stress or prenatal LPS did not have observable schizophrenia-associated behaviors, but those that received both hits had higher anxiety-like behavior.

One of the most widely used immunostimulants in the MIA literature is Poly I:C. Giovanoli et al. (2013) used Poly I:C as a first hit in a two-hit experiment, where Poly I:C was the first it and was coupled with adolescent psychological stress as the second hit to precipitate reduced sensorimotor gating and increased anxiety-like behavior, two behaviors associated with schizophrenia (Giovanoli et al., 2013). Additionally, they found an increased number of activated microglia cells in the hippocampus of two-hit animals at the end of psychological stress. Microglia cells are brain-resident macrophages that respond similarly to their somatic counterparts. Their findings, along with other studies on macrophage activation (Bilbo, 2006), suggest the activity of these cells in the mother can sensitize gestating offspring, increasing the risk of later developing schizophrenia.

C. Specific Aims

In order to optimally assess the specific role of activated T cells as a sensitizing factor in the two hit model of schizophrenia development, pregnant female C57Bl/6 mice will be randomized into either a control or T cell activation group. SEA will be delivered to pregnant dams on day E12.5 of pregnancy to induce T cell activation. Offspring will be weaned as normal and will be randomized into either a control or psychological stress condition when they are rehoused. Stress condition animals will then undergo a two-week chronic, unpredictable, mild stress (CUMS) paradigm. This will result in a two by two design of **NO ACTIVATION / NO STRESS**, **ACTIVATION / NO STRESS**, **NO ACTIVATION / STRESS**, and **ACTIVATION / STRESS**. Behavioral outcome measures focusing on spatial learning and memory, anxiety-like behavior, and sensorimotor gating will then be tested at the conclusion of psychological stress treatment. All animals will first be tested in the wRAM, followed by tests in the EPM and open field. Animals will be tested for prepulse inhibition after all other behavioral tests have been conducted. The schedule of these tests for all animals can be seen in Table 1.

With this in mind, the specific aims of this research are to:

1. **Test the effect and interaction of prenatal maternal T cell activation and postnatal stress on spatial learning and memory.**

Hypothesis 1: Animals from T cell activated mothers who have also undergone adolescent stress will have worse spatial learning and memory outcomes compared to animals who received no developmental insults.

2. **Test the effect and interaction of prenatal maternal T cell activation and postnatal stress on anxiety-like behavior.**

Hypothesis 2: Animals from T cell activated mothers who have also undergone adolescent stress will have higher levels of anxiety-like behavior compared to animals who received no developmental insults.

3. **Test the effect and interaction of prenatal maternal T cell activation and postnatal stress on sensorimotor gating.**

Hypothesis 3: Animals from T cell activated mothers who have also undergone adolescent stress will have worse sensorimotor gating compared to animals who received no developmental insults.

D. Research Design and Methods

D.1. Animals

Experiments were conducted with C57BL/6 mice. Male and female animals for breeding were purchased from The Jackson Laboratory and maintained in our vivarium (Psychology building, Busch Campus, Rutgers University New Jersey). Animals were housed up to four per cage with siblings of the same sex under a 12:12h light:dark cycle (lights on at 0530h). Food and water was available ad libitum outside of experiments. Purchased animals were allowed to acclimate for one week in our vivarium prior to mating. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health, and approved by the Rutgers Institutional Animal Care and Use Committee.

In total, 11 pregnant females (ages 6-12 weeks) gave birth to a total of 65 animals (38 males, 27 females) who were used in this study. Parental animals were between 6 and 12 weeks old at time of mating. One male animal died in the time period between repeated stress and testing in the radial arm maze. All previously collected data from this animal was removed from the study.

D.1.1. Animal Breeding

Pregnant females were generated by housing two females with one male. Males were left with females overnight and removed the next morning. Presence or absence of a sperm plug was also confirmed at this time. The day the sperm plug was identified was considered embryonic day 0.5 (E0.5). Mating female's body weight was monitored every other day to confirm a developing pregnancy. Animals confirmed as pregnant by sperm plug and progressive weight gain were randomized into either "SEA" or "saline" treatment groups on day E11.5. After treatment on day E12.5, pregnant animals were

singly housed in biohazardous temporary housing for 72 hours. Afterwards, animals were singly housed in standard shoebox cages and left undisturbed to give birth.

All pregnant females gave birth on day E19.5. On that day, pups were counted and cages were cleaned weekly to minimize distress. On post-natal day 21 (PN21), pups were sexed and randomly assigned into STRESS or NO STRESS treatment groups. Animals were group housed with siblings according to sex and assigned treatment group.

D2. Experimental Procedures

D2.1. Staphylococcal Enterotoxin A Administration

Pregnant females were injected intraperitoneally (IP) with either 250ug/kg staphylococcal enterotoxin A (SEA) (Toxin Technology) or physiological saline in a volume of 0.2ml per injection per animal on day E12.5. All injections were given between 1200h and 1400h.

D2.2. Pubertal Repeated Stress Administration

Offspring were randomized into STRESS and NO STRESS treatment groups on day PN21. Animals in the “stress group were exposed to 14 days of repeated stress, starting on PN34 and ending on PN47. Stressors lasted for two hours each and were administered at 1300h and 1500h each day. Stressors are indexed in detail in the following sections. Table 2 includes the schedule of stressors the animals experienced. The schedule of stressors was generated by randomizing each time block before the experiment began. Our stress paradigm was created to be extremely mild, with individual stressors inspired by previous work in the literature (Li et al., 2015; Liu, Wang, Wang, Li, & Ji, 2015).

Over the course of repeated stress, animal weights were recorded daily, before stress administration for that day. Food and water was also weighed per cage. Food consumption per animal per day was calculated as the change in food weight over the previous 24 hours divided by the number of animals in the cage.

D2.2.1. Stressors

D2.2.1.1 Restraint

Animal restrainers were made using 50ml Falcon conical tubes. Each tube had 80 holes drilled into the sides to allow for air passage. Animals were inserted into the tubes head-first, with the lid closed behind them. Tubes were placed so that the animal was right side up (feet towards the bottom of the cage) back in their home cage.

D2.2.1.2. Bright Light / Isolation

Animals were singly housed in standard shoebox cages for the duration of this stressor. Cages were deprived of food, water, and nesting material to reduce the potential for shadows. Cages were housed under bright fluorescent lights for the duration.

D2.2.1.3. Wet Bedding

Standard corn cob bedding was thoroughly soaked in water for five minutes. Afterwards, excess water was completely drained and wet bedding was added to empty, clean standard shoebox cages. Animals were placed in these wet bedding cages with their cage mates, and food and water was available ad libitum for the duration.

D2.2.1.4. White Noise

Animal home cages were placed inside a San Diego Instruments acoustic startle chamber (1 cage per chamber). After a two minute acclimation period, random

intermittent pulses of noise were presented that varied in intensity, duration, and intertrial interval. The tones were either 40db, 80db, or 120db, for a duration of either 0.5 seconds, 1 second, or 2 seconds. The intertrial intervals were either 15 seconds, 30 seconds, or 45 seconds. The noise was presented over the two hour duration.

D3. Behavioral Tasks

Water radial arm maze, elevated plus maze, and open field data collection took place in testing areas enclosed by blue plastic curtains and were digitally recorded by an overhead webcam connected to a laptop running AnyMaze animal tracking software (Stoelting). For each test, a single experimenter remained in the room, outside of the enclosure, while data was being collected. For prepulse inhibition, animals were placed into the acoustic startle chambers. After setting the experimental program, the experimenter left the room for the duration of the test.

D3.1. Cognitive Measures

D3.1.1. Water Radial Arm Maze (wRAM)

Water mazes have been used extensively in assessing learning and memory across species and benefit from having high reliability, minimal pre-training of animals, and validity as a measure of hippocampus-dependent spatial learning (Vorhees & Williams, 2006). While the Morris water maze was originally proposed, after piloting the experiment and consulting the recent literature, the apparatus was changed to the wRAM. The wRAM has been demonstrated as more sensitive to teratogenic effects compared to the Morris water maze (Akaike, Ohno, Tsutsumi, & Omosu, 1994). The maze consists of eight 15cm wide arms (50cm long) connected to a central hub (30cm radius) . The entire maze is constructed of opaque black polyurethane with chemically welded seams. The maze was filled with water made opaque with white, powdered,

non-toxic tempera paint. The water was kept between 20 and 22 degrees Celsius. The the walls of the wRAM extended 10cm above the water's surface. Subjects were unable to escape by using the walls during testing, nor were they able to touch the bottom of the apparatus while swimming. During hidden platform trials, a clear, rectangular acrylic platform measuring 15cm by 10cm was submerged 1cm below the surface of the water in one arm of the maze (arm 8). For flag-training trials, a black and white paper flag was attached to the submerged platform to create an intramaze cue. Flag-training trials were used to expose subjects to an escape condition within the maze. Throughout the entire wRAM experiment, the platform remained in arm 8.

The maze was placed in an environment with many distal cues. There were four intentional distal cues, each corresponding to a cardinal direction around the maze. A blue wall with white stripes to the south, a plain blue wall to the east, a white wall with shelves to the north, and a plain white wall to the west. The maze remained unmoved and the environment untouched for the duration of the experiment.

Following the standard hippocampus-dependent spatial learning paradigm, animals were first flag trained for two days. The first day of flag training was performed on day PN58. Each day, each animal performed four trials in the maze. Each trial, the animal started in a randomized arm. The starting arm positions were randomized before the start of the experiment. Latency to find the platform was recorded to a maximum of 60 seconds. If after 60 seconds the animal did not find the platform, the experimenter placed the animal onto the platform for 15 seconds before returning it to a cage. This was scored as the maximum of 60 seconds. After a 60 second intertrial interval (ITI), the same animal was placed into the maze again for the second trial. This was repeated for a total of four trials per animal.

After the two days of flag training, the animals were given two days rest before hidden platform training began. Hidden platform trials were performed the same as flag training trials, with the exception that the flag was no longer being present in the maze. Hidden platform training lasted for seven consecutive days (Days 1-7, four trials per day).

On the day 8, a probe trial were conducted. For this trial, the hidden platform was removed from the maze. Animals were allowed to swim the maze for 60 seconds. After their 60 second trial, the animal was returned to their cage. After all animals had performed this probe. Time spent (seconds) in the target arm (arm 8, where the platform had previously been) and latency (seconds) to enter target arm were measured.

For all segments of the wRAM, total distance traveled (meters), duration of time spent in the maze (seconds), latency to find the hidden platform (seconds), and speed (meters/second) were recorded. Further distance traveled before finding the platform, longer duration in the maze, and higher latency to find the platform indicated worse acquisition performance in the hidden platform trials. Less time in the target arm, and longer latency to first enter the target arm indicated worse spatial memory in the probe trial.

D3.2. Anxiety Measures

D3.2.1. Elevated Plus Maze (EPM)

The elevated plus maze is a widely used apparatus for measuring anxiety-like behavior in rodents. The maze consists of two walled arms and two exposed arms, and the animal's behavior in this task reflects a conflict between preference for protected areas (the closed arms), and the motivation to explore novel environments (the open arms) (Walf & Frye, 2007). Each animal was tested in the EPM for one trial of five

minutes, starting in the center zone of the maze. Animals were tested on day PN73. At the end of the trial, the animal was returned to its home cage. Recorded measures include the time in open and closed arms, as well as the center area, number of entries into closed and open arms, the total distance traveled in the maze, and the ratio of time spent in the open arms to closed arms. More time spent in the closed arms, and less time spent in the open arms indicated higher anxiety-like behavior.

D3.2.2. Open Field +/- Novel Object (OF)

The open field +/- novel object task measures exploratory behavior in the absence and presence of a novel object. The open field apparatus measures 63 x 57 x 28cm and is made of opaque plexiglass. Animals were tested in the open field on day PN73. Each animal was tested for one trial of five minutes in the empty open field (open field – novel obect) and their exploratory behavior was recorded. At the end of the five minute trial, the animal was cordoned to the start corner (the corner of the maze closest to the exit of the testing room), and a novel object (a metal cylinder measuring 6cm by 15cm) was placed in the center of the maze. After the object was placed, the animal was allowed another five minutes to explore. Total exposure in the open field apparatus was 10 minutes per animal (Boersma et al., 2015). After the 10 minutes, the animal was returned to its home cage. Total distance traveled, time spent in perimeter and open zones, and distance to the novel object were measured. Smaller ratio of time spent and further mean distance from the novel object will indicate greater anxiety-like behavior.

D3.3. Measures of Schizophrenia-Associated Behavior

D3.3.1. Prepulse Inhibition (PPI)

Clinical studies in patients with schizophrenia have shown that the disease impairs sensorimotor gating (Kohl, Heekeren, Klosterkotter, & Kuhn, 2013). Prepulse

inhibition of the startle reflex is a mechanism of measuring sensorimotor gating and refers to the ability of a weak sensory stimulus to inhibit the response to a closely timed strong sensory stimulus (Kumari, Soni, Mathew, & Sharma, 2000). Impaired sensorimotor gating is made evident by the decreased ability for the weaker stimulus to inhibit the response to the stronger stimulus (Braff & Geyer, 1990).

A behavioral model of prepulse inhibition designed for mice to measure schizophrenia-associated behavior has been utilized. Each animal was placed into a San Diego Instruments acoustic startle chamber on day PN 76. The chambers consist of an acrylic tube (which holds the animal in an unrestrained manner) which is mounted on an acrylic plate, under which is attached an accelerometer. The accelerometer measured the inflection of the animal/tube in response to acoustic stimulation. White noise was transmitted to the box via a speaker in the top of the isolation compartment. Pulses of white nose of 120db were considered the strong pulse, intended to elicit highest level of startle.

The PPI test consisted of several different segments. “Pulse alone” trials (S) consisted of 20, 40 millisecond (ms) 120db tones, delivered at an ITI of 60 seconds. Startle response from these trials served as a positive control for startle. Five S trials were delivered at the beginning and end of the PPI paradigm, with the remaining 10 delivered randomly throughout the paradigm. “Prepulse alone” trials (p) consisted of 10, 40ms of either 62db, 65db, or 68db white noise and served as a negative control (prepulse tones should not be inherently startling – a startle response to these tones would have been evidence that our prepulse was too intense). These p trials were delivered randomly throughout the paradigm. Test trials (PPI_S) had both a prepulse and a pulse, separated by an inter-pulse interval of 100ms. Animals were exposed to 10

PPI_S trials of each prepulse intensity (30 trials total), distributed randomly throughout the test paradigm.

Percent scores for amount of prepulse inhibition were calculated as: %PPI = 100 * (S – PPI_S) / S. Scores closer to 0% will be evidence of less prepulse.

D3.4. Data Analysis

Prior to assessing the hypotheses, frequencies for measured variables were calculated. For each variable, measures of central tendency and dispersion were calculated, as well the presence of any outliers were assessed.

For normally distributed data, multiple regression was used to control for extraneous variables. These are reported as unstandardized beta coefficients (b) with corresponding F statistic. For longitudinal data, repeated measures ANOVA was used to test for equality of means over time. These are reported as F statistics. For count data, Poisson general linear model (GLM) was used to account for the non-normal distribution. These are reported as unstandardized beta coefficients with corresponding Z values. When appropriate, post-test pairwise comparisons were performed with either Tukey's Honest Significant Difference (Tukey HSD), or Bonferroni adjustment. Significance was set at $p < 0.05$.

E. Results

E1. Pubertal Repeated Stress

E1.1. Body Weight

To determine if maternal SEA treatment affected offspring weight at the start of stress administration (PN34), animal weights were collected. There was no statistically significant effect of treatment ($F(3, 59) = 2.607$, p =n.s) , though males were significantly heavier than females for all treatment groups ($b = 2.45$, $F(1, 59) = 35.331$, $p < 0.5$) (Figure 1).

Following stress administration (PN47), weights were compared to determine if stress treatment had an effect on final body weight. Animals that underwent postnatal stress gained significantly less weight compared to non-stressed animals (b = -0.73, $F(1, 59) = 5.65$, $p < 0.05$). We did not observe an effect of SEA treatment ($F(1, 59) = 3.79$, p=n.s) though males continued to be significantly heavier than females for all treatment groups ($b = 3.98$, $F(1, 59) = 154.64$, $p < 0.05$) (Figure 2).

To determine if stress treatment resulted in changes in food consumption, food weight was recorded for all days during the stress protocol. Food consumption per animal per day can be seen in Figure 3a. Across the 14 days of stress, animals in the stress treatment group had a reduction in food consumption ($F(1, 24) = 8.62$, $p < 0.05$). This change in food intake can be seen in Figure 3b, with animals in the stress condition eating less food on day 14 and animals in the non-stress condition eating more food.

E2. Water Radial Arm Maze (wRAM)

All animals were run in the wRAM for 10 days total (2 flag training days, 7 hidden platform days, and one probe day). Flag training started on day PN58, which was 11

days after repeated stress ended. The final day of wRAM testing (probe trials) was PN69.

E2.1. Acquisition Training: Duration of Time Spent in Maze

After two days of flag training, animals performed seven days of hidden platform (acquisition) training in the wRAM (Figure 4). There was a significant effect of day ($F(1, 56) = 115.21$, $p < 0.05$); all treatment groups spent less time in the maze as the test progressed. There was also a significant main effect of treatment ($F(3, 56) = 3.45$, $p < 0.05$). Pairwise comparisons with a bonferroni adjustment revealed a significant difference between the SEA-No Stress group and the Saline-No Stress group ($p < 0.05$), the Saline-Stress group ($p < 0.05$), and the SEA-Stress group ($p < 0.05$). As seen in Figure 4, the SEA-No Stress animals spent significantly longer in the wRAM compared to the other treatment groups across all days. There was no sex effect ($F(1, 56) = 0.001$, p = n.s) or treatment by sex interaction ($F(3, 56) = 0.366$, p =n.s).

E2.1.1. Acquisition Training: Latency to Find the Hidden Platform

Some animals never found the hidden platform in the 60-second timeframe of the trial. When excluded, the remaining data allowed for the analysis of latency to find the hidden platform (Figure 5). There continued to be a significant effect of day ($F(1, 48) = 8.14$, $p < 0.05$) and of treatment ($F(3, 48) = 3.43$, $p < 0.05$). Pairwise comparisons with a bonferroni adjustment revealed a significant difference between the SEA-No Stress group and the Saline-No Stress group ($p < 0.05$) and the Saline-Stress group ($p < 0.05$), and a marginally significant difference between the SEA-No Stress group and the SEA-Stress group ($p = 0.055$). As seen in Figure 5, the SEA-No Stress animals that found the hidden platform took significantly longer to find it compared to the other treatment

groups. There was no sex effect ($F(1, 48) = 0.047$, p =n.s) or treatment by sex interaction ($F(3, 56) = 1.571$ p =n.s).

E2.2. Acquisition Training: Fail Errors

A trial where the animal failed to find the hidden platform after 60 seconds was scored as a "fail error". The total number of fail errors over the seven days of acquisition training was calculated for each animal. Using a Poisson general linear model to account for the non-normal distribution of the data, a main effect of SEA on number of fail errors was detected ($b = 0.94$, $Z = 3.76$, $p < 0.05$), with animals from SEA treated mothers having making significantly more fail errors (Figure 6). This means offspring from SEA treated mothers had more trials where they did not find the platform in the allotted 60 seconds compared to offspring from saline treated mothers. An effect of sex was not detected (b = 0.08, Z = 0.44, p=n.s).

E2.3. Acquisition Training: Distance Traveled in Maze

Figure 7 shows the total distance traveled over the duration of acquisition training. There was a main effect of treatment on distance traveled ($F(3, 56) = 3.08$, $p < 0.05$). Pairwise comparisons with a bonferroni adjustment revealed a significant difference between the SEA-No Stress group ($p < 0.05$) and the Saline-No Stress group ($p < 0.05$), where the SEA-No Stress animals traveled significantly further in the maze.

Additionally, there was a statistically significant sex by day interaction ($F(1, 56) = 4.17$, $p < 0.05$), where female animals significantly reduced distance traveled in the maze over time. This can also be seen in Figure 7.

E2.4. Acquisition Training: Swim Speed

To determine if latency to find the hidden platform was influenced by idle activity in the maze (e.g. floating in one arm), swim speed was calculated for each animal, and can be seen in Figure 8. Speed did not appear to differ between treatment groups ($F(3, 56) = 1.42$, p=n.s), across days ($F(1, 56) = 0.41$, p=n.s), or by sex ($F(1, 56) = 0.86$, p=n.s).

E2.5. Spatial Memory Testing: Probe Trials: Time Spent in Target Arm

Twenty-four hours after the final day of acquisition training, each animal was allowed to freely swim the maze for one trial of 60 seconds. During this probe trial, the hidden platform was completely removed from the maze. Figure 9 shows total time spent in the target arm (arm 8, which previously had the hidden platform). There was a significant main effect of treatment on time spent in the target arm ($F(3, 44) = 3.64$, $p < 0.05$). Tukey's Honest Significant Difference posttest revealed a statistically significant difference between the Saline-No Stress and SEA-Stress groups ($p < 0.05$), meaning SEA-Stress animals spent significantly less time in the target arm compared to Saline-No Stress control animals. An effect of sex was not detected ($F(1, 44) = 0.53$, p=n.s).

E2.5.1 Spatial Memory Testing: Probe Trials: Latency to First Enter Target Arm

Figure 10 shows the latency to first enter the target arm. There was a significant SEA by stress interaction ($b = 0.65$, $Z = 4.41$, $p < 0.01$), where SEA-Stress animals took significantly longer to make their first entry into the target arm. There was also a marginally significant main effect of sex ($b = 0.15$, $Z = 1.89$, $p = 0.58$). Adding sex to the interaction term, there was a significant three-way interaction of SEA by stress by sex ($b = 0.91$, $Z = 2.7$, $p < 0.01$). This can be seen in Figure 10, where male SEA-Stress animals take significantly longer to enter the target arm for the first time. Female SEA-Stress animals enter the arm just as fast as the other treatment groups.

E3. Elevated Plus Maze (EPM)

All animals were run for one five minute trial in the elevated plus maze. EPM testing was performed on day PN73, which was four days after the final day of wRAM testing.

E3.1. Distance Traveled

Figure 11 shows total distance traveled in the elevated plus maze. There was a significant main effect of stress on distance traveled (b = 2.6, F(1, 59) = 15.35, $p < 0.05$), with both Saline-Stress and SEA-Stress treatment groups traveling significantly further in the EPM compared to Saline-No Stress and SEA-No Stress animals.

E3.2. Arm Preference Measures

Figure 12 shows the total number of open arm entries over the EPM trial. Neither an effect of maternal SEA (F(1, 59) = 2.95, p=n.s) nor stress (F(1, 59) = 2.70, p=n.s) was detected. Similarly an effect of sex was not detected (F(1, 59) = 3.80, p=n.s).

E4. Open Field +/- Novel Object (OF +/- NO)

Following the EPM on PN73, animals were run for 10 minutes in the Open Field (OF). For five minutes, animals were allowed to freely explore the maze. Afterwards, a novel object was placed in the center of the maze and animals were given an additional five minutes to explore.

E4.1. Open Field without Novel Object

E4.1.1. Distance Traveled

Figure 13 shows the total distance traveled in the open field, without an object. There was a significant main effect of stress on distance traveled (b = 2.67, F(1, 61) =

4.62, $p < 0.05$), with both Saline-Stress and SEA-Stress treatment groups traveling significantly further in the OF without object compared to Saline-No Stress and SEA-No Stress animals.

E4.1.2. Time in Center Area

Figure 14 shows the total time spent in the center area of the open field, without an object. There was a main effect of maternal SEA treatment on time spent in the center (b = -11.93, F(1, 61) = 8.60, $p < 0.05$), meaning offspring from SEA treated mothers spent significantly less time in the open area of the maze compared to the Saline treatment animals.

E4.2. Open Field with Novel Object

E4.2.1. Distance Traveled

Figure 15 shows the total distance traveled in the open field, with an object. There were no statistically significant effects of either maternal SEA treatment (F(1, 61) = 0.96, p=n.s) or stress treatment (F(1, 61) = 0.13, p=n.s).

E4.2.2. Time in Center Area

Figure 16 shows the total time spent in the center area of the open field, with an object. There was a significant main effect of maternal SEA treatment on time spent in the center (b = -18.07, F(1, 61) = 4.44, p <0.05), meaning offspring from SEA treated mothers spent significantly less time in the open area of the maze compared to the Saline treatment animals.

E4.3. Open Field: Comparison between No Object and Object Trial

E4.3.1. Distance Traveled

Figure 17 shows the distance traveled in the open field in the no object trial and the object trial. There was a significant main effect of object trial on total distance traveled ($F(1, 63) = 86.88$, $p < 0.05$), meaning after the novel object was introduced to the open field, all animals traveled less total distance in the maze.

E4.3.2. Time in Center Area

Figure 18 shows the time spent in the open area of the field in the no object trial and object trial. There was a significant main effect of object trial on time spent in the center area ($F(1, 63) = 77.48$, $p < 0.05$), meaning all animals spent more time in the open part of the maze after the novel object was introduced.

E5. Prepulse Inhibition

All animals were assessed for prepulse inhibition on PN76, which was two days after EPM and OF testing. Figure 19 shows the percent prepulse inhibition for each treatment group over the three prepulse intensities tested. There were no significant effects of treatment ($F(3, 59) = 0.20$, p=n.s) or prepulse intensity ($F(2, 120) = 1.69$, p=n.s) detected on percent prepulse inhibition.

F. Discussion

It was hypothesized that maternal immune activation coupled with adolescent stress would generate animals with deficits in spatial learning and memory, an increase in anxiety-like behavior, and a deficit in prepulse inhibition. Animals receiving only one stressor, either maternal SEA treatment or adolescent stress, would show less, if any, behavioral deficit. Some data collected supports this original hypothesis, while more supports an effect of maternal immune activation alone, with or without the addition of adolescent stress.

SEA-Stress animals spent less time in the target arm following 7 days of training in the water radial arm maze, supporting hypothesis 1 which predicted that animals in the two-hit condition would display deficits in spatial memory (Figure 10). However, during the training phase in the maze, we found SEA-No Stress animals traveling significantly further in the maze for all days, suggesting these animals gained less insight on maze escape from the two prior days of flag training (Figure 4, Figure 8a & 8b). SEA-No Stress animals also took longer to find the hidden platform for all test days (Figure 5). These animals learned at the same rate as the other three treatment groups, meaning these animals were capable of experiential learning over time. We did not see deficits in the two-hit animals over the course of maze acquisition.

Furthermore, offspring from SEA treated mothers made more fail errors in the water radial arm maze, meaning they spent the full 60 seconds in the maze without finding the platform. This provides additional evidence outside of my specific aims for an effect of maternal SEA treatment on spatial learning deficits

Two-hit animals did not exhibit more anxiety-like behavior in either the elevated plus maze or the open field +/- novel object task. This evidence does not provide

support for hypothesis 2. However, there was a main effect of maternal SEA-treatment on time spent in the center of the open field, both with and without the novel object (Figures 15 & 17). Animals from SEA treated mothers spent less time in the center area of the maze compared to animals from saline treated mothers. These results are not surprising given the previous MIA work done in the C57BL/6 strain (Enayati et al., 2012). In their work, Enayati et al., did not detect anxiety-like behavior from MIA offspring in C57BL/6. Compared to their work, our results are mixed. Though data from the elevated plus maze does not support an increase in anxiety-like behavior, data from our open field experiment does. As Enayati et al. did not test their animals in the open field for anxiety-like behavior, this may be one test where C57BL/6 show an anxiety-like phenotype.

Two-hit animals also did not exhibit deficits in sensorimotor gating (Figure 20). All animals had prepulse inhibition scores near 50%. This result was most surprising, as most MIA literature report deficits in prepulse inhibition for offspring from treated mothers (Giovanoli et al., 2013; Zhang & van Praag, 2015). While this data does not provide support for hypothesis 3, this test did not include a positive control. Without this positive control, is hard to draw a definitive conclusion from this prepulse inhibition data, as it is possible our equipment was incapable of detecting a different in prepulse inhibition.

Additionally, it is possible that maternal T cell activation cause different effects to offspring that does not confer a loss in prepulse inhibition. As this is the first report using SEA in a MIA paradigm, it just may be that maternal T cell activation does not produce a PPI deficit in resulting offspring. In this way, we cannot compare directly to the existing literature. However, additional tests including Poly I:C or LPS control would help further elucidate the effect of maternal T cell activation on offspring prepulse inhibition.

There is a precedent in the literature to support the effect of maternal immune activation alone in the generation of behavioral deficits. Richetto, Calabrese, Meyer, and Riva (2013) found offspring to Poly I:C treated mothers alone had spatial working memory deficits that were independent of postnatal maternal behavior (Richetto, Calabrese, Meyer, & Riva, 2013). Zhang and van Praag observed similar results in the T-maze (Zhang & van Praag, 2015). Additionally, work has been done using lipopolysaccharide (LPS) and individual pro-inflammatory cytokines such as Il-1 beta to probe behavioral deficits in offspring (Boksa, 2010; Deverman & Patterson, 2009). All of these models have been studied as a single experimental treatment and have caused psychopathology in the offspring.

There are some improvements that could be made to this described method. The chronic stressor paradigm used in this thesis is very mild in comparison to the literature. For example, Erburu et al. (2015) used 10 days of chronic social defeat stress, which involves cohousing with aggressive animals with attack latencies less than 30 seconds (Erburu et al., 2015). Our mild stressor paradigm did not contain any social stressor or physical trauma. Deng et al. (2015), used a “Chronic Unpredictable Mild Stress (CUMS)” paradigm, which included overnight illumination, tail pinch, 10 hours of white noise, and 12 hours of both cage tilt and wet bedding (Deng et al., 2015). By comparison, our stress paradigm used only 2 hours of wet bedding, cage tilt, or white noise, when applicable. At most, animals were exposed to four hours of stress per day over the 14-day paradigm. It may be that our adolescent stress was too weak to generate measurable effects in the offspring.

Additionally, our maternal SEA treatment may have been too strong to allow for adolescent stress to make a measurable difference. Giovanoli et al. (2013) describes unmasking a latent phenotype that was generated by a suboptimal dose of Poly I:C

given to the pregnant dam (Giovanoli et al., 2013). Alone, this dose of Poly I:C was not enough to generate an effect in offspring. However, in previous work (Meyer, Feldon, Schedlowski, & Yee, 2006), they have shown an effect with a larger dose of Poly I:C alone. This may be relevant to my current line of research – the maternal SEA treatment described in this thesis may be such a strong hit, that psychopathology develops regardless of a second hit. Future studies may benefit from titrating maternal SEA exposure to find a dose that generates a latent phenotype made observable by adolescent psychological stress.

Despite these weaknesses, there are several strengths that are notable. Firstly, this is the first report of T cell specific maternal immune activation leading to behavioral effects in adult offspring. These effects were observable in both a spatial learning test as well as a test for anxiety-like behavior. This work provides evidence for a potential role of activated T cells during pregnancy in the development of psychopathologies in adult offspring. Additionally, this work will serve as a foundation to subsequent studies on the cellular and molecular processes underlying maternal immune involvement in fetal development.

Secondly, this study focused on both male and female offspring. While previous literature has mainly focused on male offspring only, my approach allowed analysis of the full litter from each treated mother. In some cases, sex differences were observed. These results may support additional research into how male and female offspring are affected in different ways by maternal immune activation.

It is important that future research identify specific parts of the immune system that are particularly relevant for the development of behaviors associated with schizophrenia. By using a bacterial superantigen, we specifically targeted T cells for maternal immune activation and found interesting results in both spatial learning and

anxiety-like behavior. Nevertheless, future research should continue to identify those immune cells of the mother that may alter fetal development. Additionally, future research must continue to focus on both male and female offspring when studying the effects of maternal immune activation. With NIH guidelines concerning the study of both males and females on the horizon, this study is ahead of the curve. Finally, it is critical to further explore the finding that maternal immune activation is associated with worse outcomes in spatial learning. Specifically, future research should examine this in other spatial learning tests and whether this effect holds over longer time periods.

G. Tables and Figures

Table 1: *Behavioral test schedule for all animals.*

Test day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Age	**PN58**	**PN59**	**PN60**	**PN61**	**PN62**	**PN63**	**PN64**	**PN65**
Behavioral test	wRAM	wRAM			wRAM	wRAM	wRAM	wRAM
Test day	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Age	**PN 66**	**PN67**	**PN68**	**PN69**	**PN70**	**PN71**	**PN72**	**PN73**
Behavioral test	wRAM	wRAM	wRAM	wRAM				EPM & OF
Test day	**17**	**18**	**19**					
Age	**PN74**	**PN75**	**PN76**					
Behavioral test			PPI					

Table 2: *Randomized stressor schedule.*

Day	Stressor 1 (time period:13:00-15:00h)	Stressor 2 (time period:15:00-17:00h)
Baseline	None	None
Day 1	Bright Light	Bright Light
Day 2	White Noise	Restraint
Day 3	Damp Bedding	Damp Bedding
Day 4	Restraint	Bright Light
Day 5	Damp Bedding	Damp Bedding
Day 6	Bright Light	Bright Light
Day 7	Restraint	White Noise
Day 8	Bright Light	Bright Light
Day 9	Restraint	Restraint
Day 10	Damp Bedding	Damp Bedding
Day 11	White Noise	Restraint
Day 12	Restraint	Restraint
Day 13	Damp Bedding	Damp Bedding
Day 14	Bright Light	Restraint
Day 15	None	None

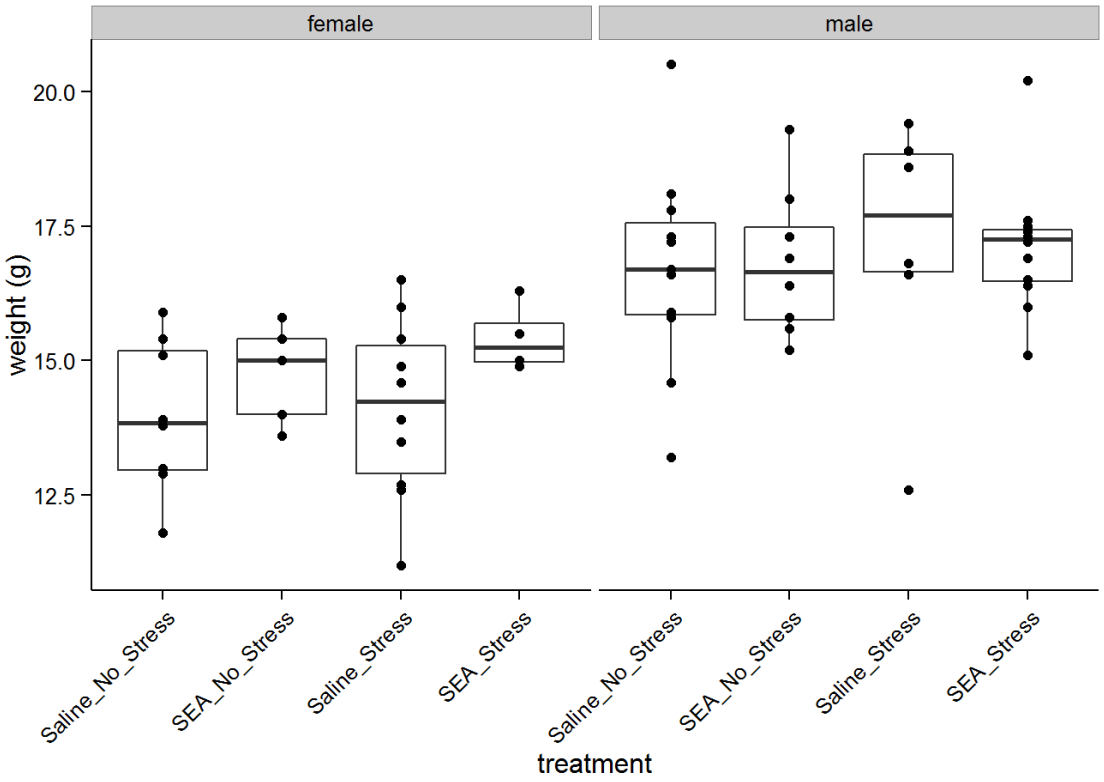

Figure 1. Animal body weight on day 0 of stress, faceted by sex. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Data split into female and male animals. Female animals across all treatment groups weighed less than male animals (b = 2.45, F(1, 59) = 35.331, p < 0.5).

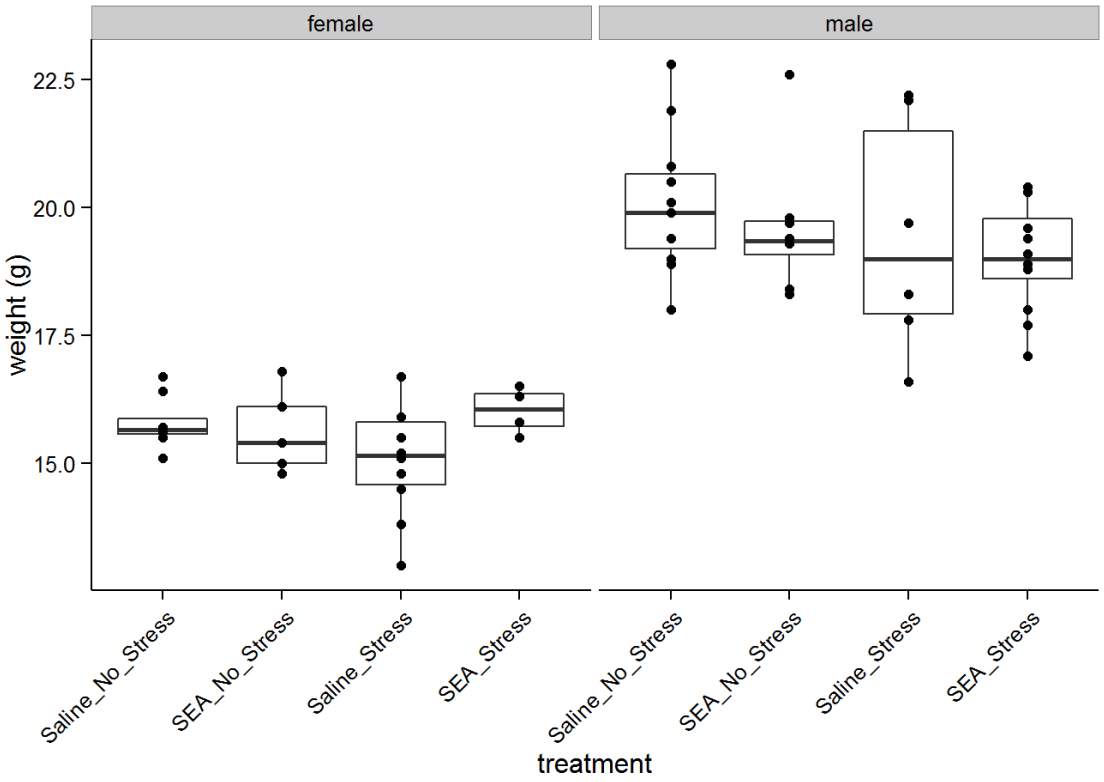

Figure 2. Animal body weight on day 15 of stress, faceted by sex. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Data split into female and male animals. Female animals across all treatment groups weighed less than male animals (b = 3.98, F(1, 59) = 154.64, $p < 0.05$).

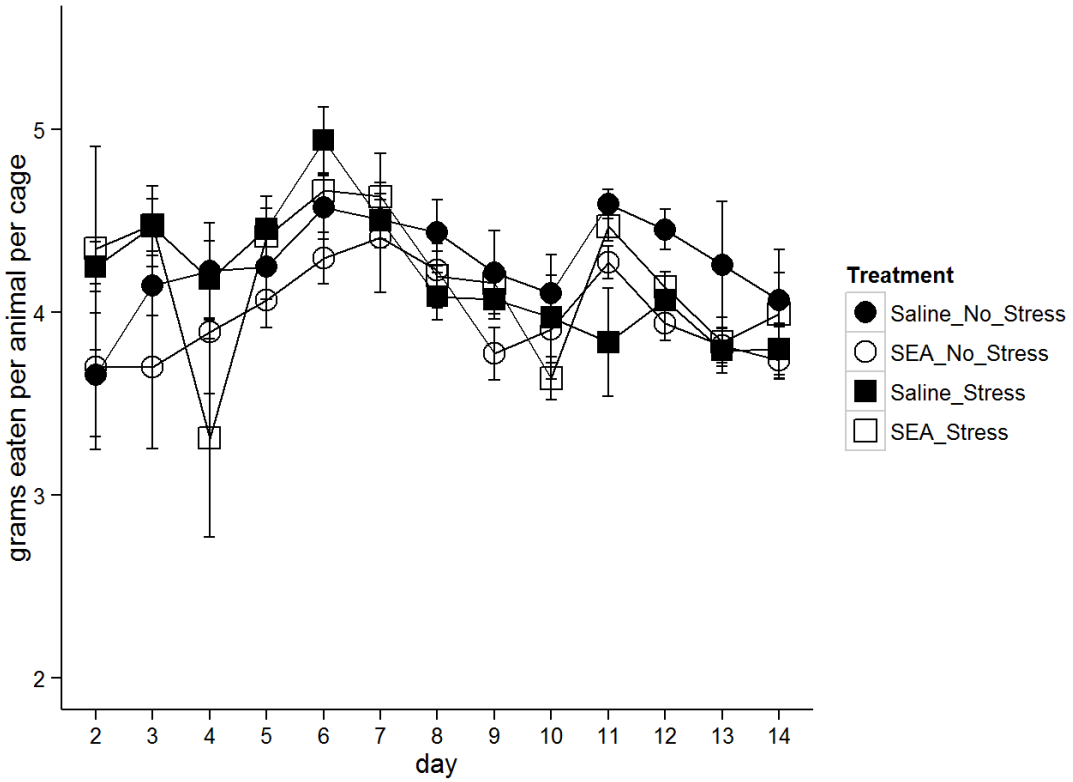

Figure 3a. Grams of food eaten per animal per cage. Data expressed as mean grams of food eaten per cage per day by treatment +/- standard error of the mean (SEM). Significant stress by day interaction, $F(1,28) = 6.18$, $p < 0.05$, with stress treatment animals eating less food over days.

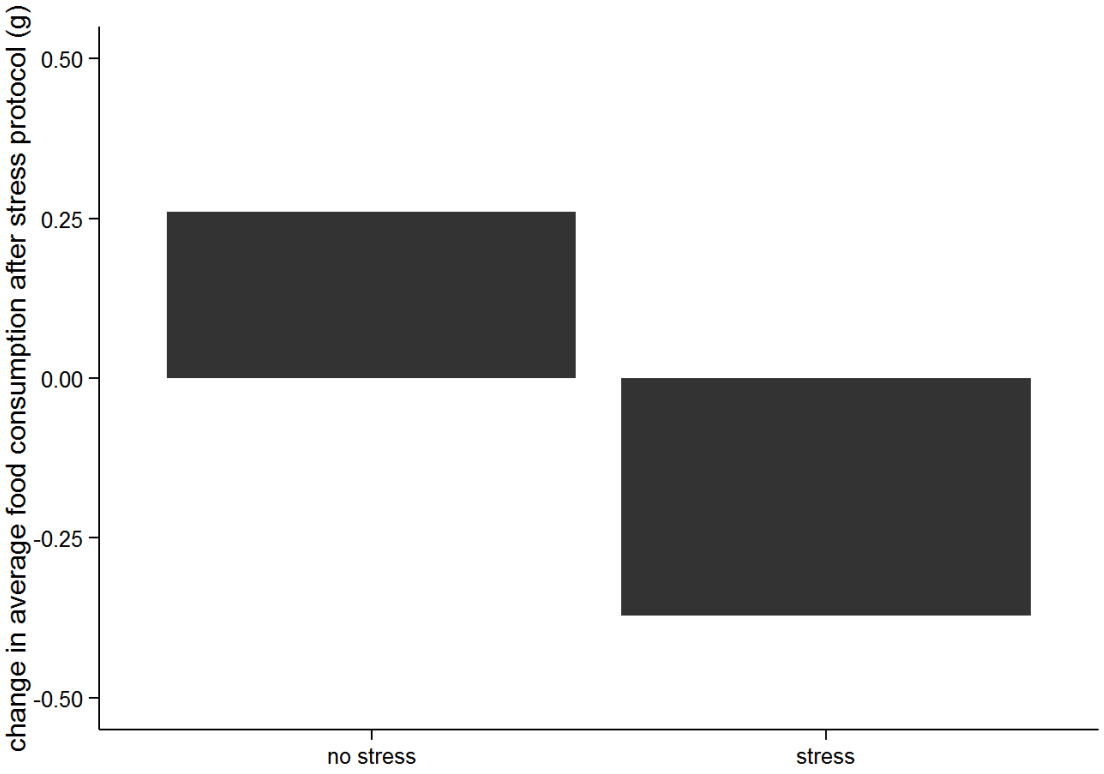

Figure 3b. Change in average food consumption after the stress protocol. Data expressed as mean change in grams of food eaten, for animals that were either stressed or not stressed. Significant stress by day interaction, $F(1,28) = 6.18$, $p < 0.05$, with stress treatment animals eating less food by the end of stress protocol.

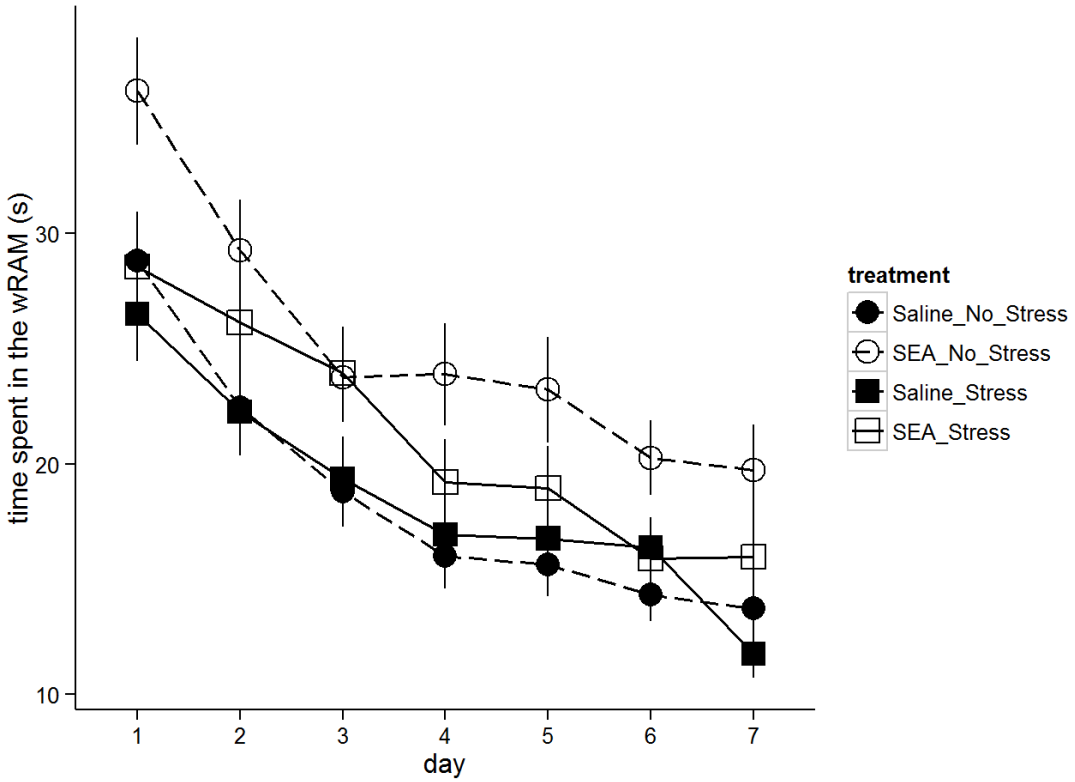

Figure 4. Duration of time spent in the water radial arm maze. Data expressed as mean duration per treatment per day +/- standard error of the mean (SEM). Significant main effect of day ($F(6, 56) = 115.21$, $p < 0.01$) and treatment ($F(3, 56) = 3.45$, $p < 0.05$). Pairwise comparisons with Bonferroni adjustment. For all days, SEA-No Stress animals spent more time in the maze.

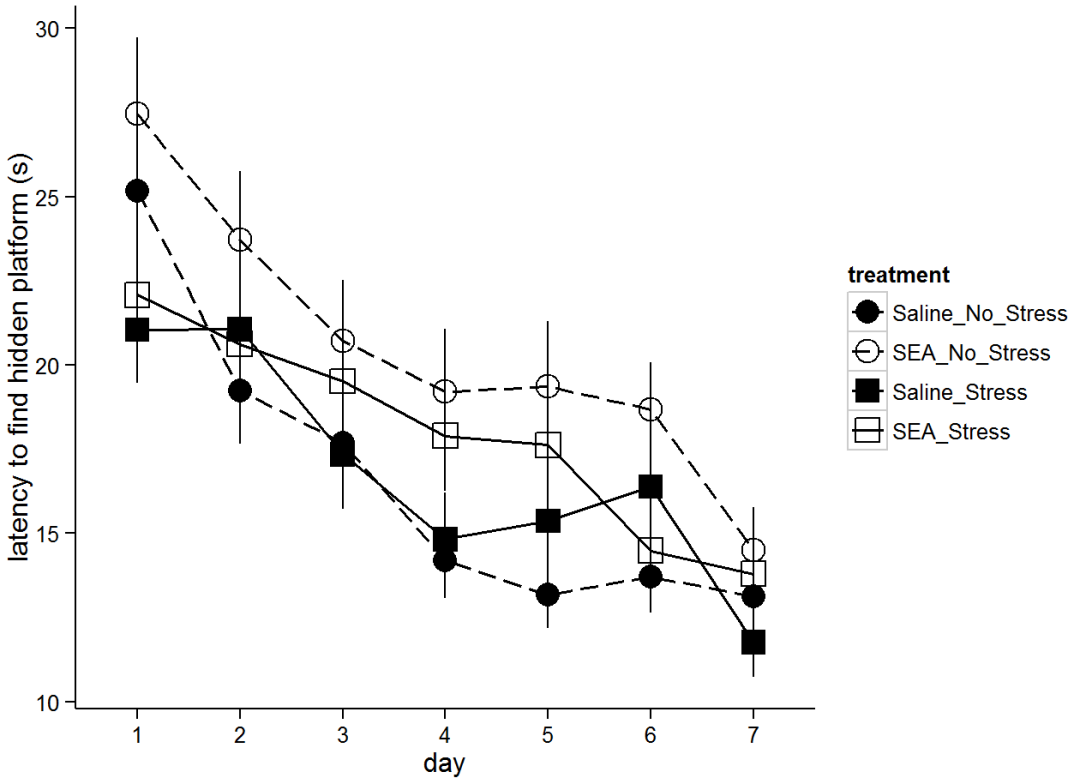

Figure 5. Latency to find the hidden platform, considering only those animals that found the platform in the allotted time. Data expressed mean duration per treatment per day +/- standard error of the mean (SEM). Significant main effect of day $F(6, 48) = 8.14$, $p < 0.01$) and of treatment ($F(3, 48) = 3.43$, $p < 0.05$). Pairwise comparisons with Bonferroni adjustment between all groups. For all days, SEA-No Stress animals spent more time in the maze compared to all other groups.

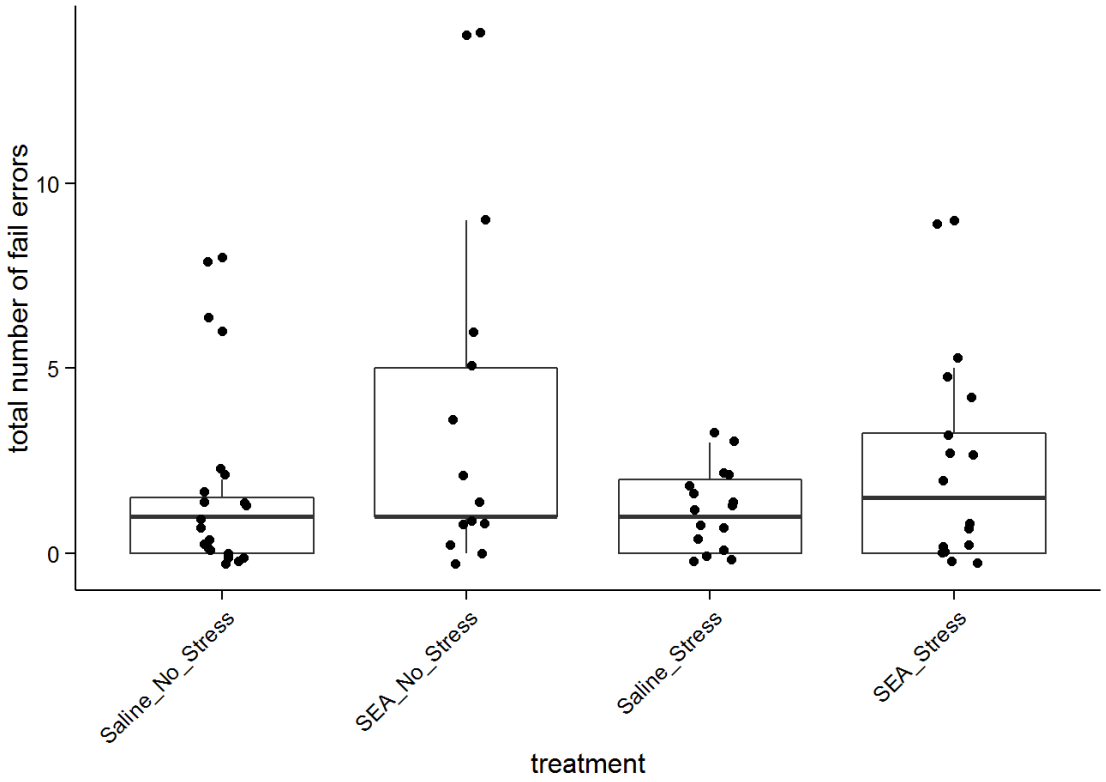

Figure 6. Number of fail errors made in the water radial arm maze. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Significant main effect of maternal SEA treatment (b = 0.94, Z = 3.76, p < 0.01), with SEA treatment animals making more fail errors.

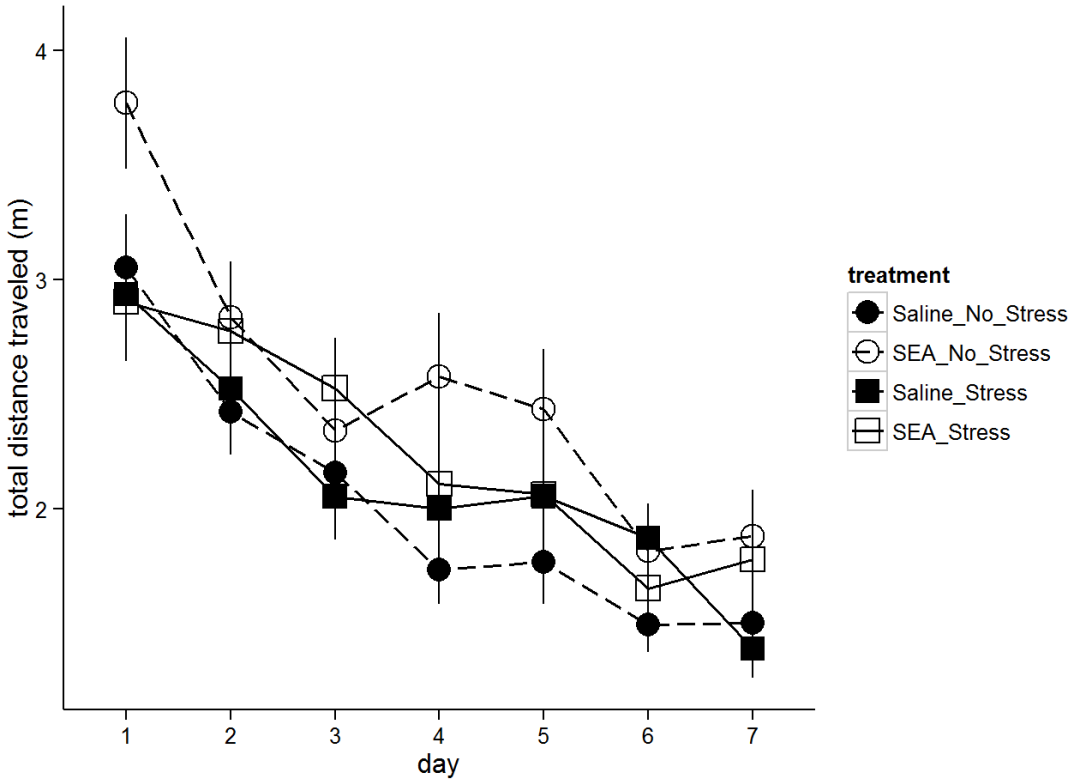

Figure 7. Distance traveled in the water radial arm maze. Data expressed as mean distance traveled per treatment per day +/- standard error of the mean (SEM). Significant main effect treatment on distance traveled ($F(3, 56) = 3.08$, $p < 0.05$). Pairwise comparisons with Bonferroni adjustment between all groups. For all days, SEA-No stress animals traveled significantly further than Saline-No stress animals in the wRAM.

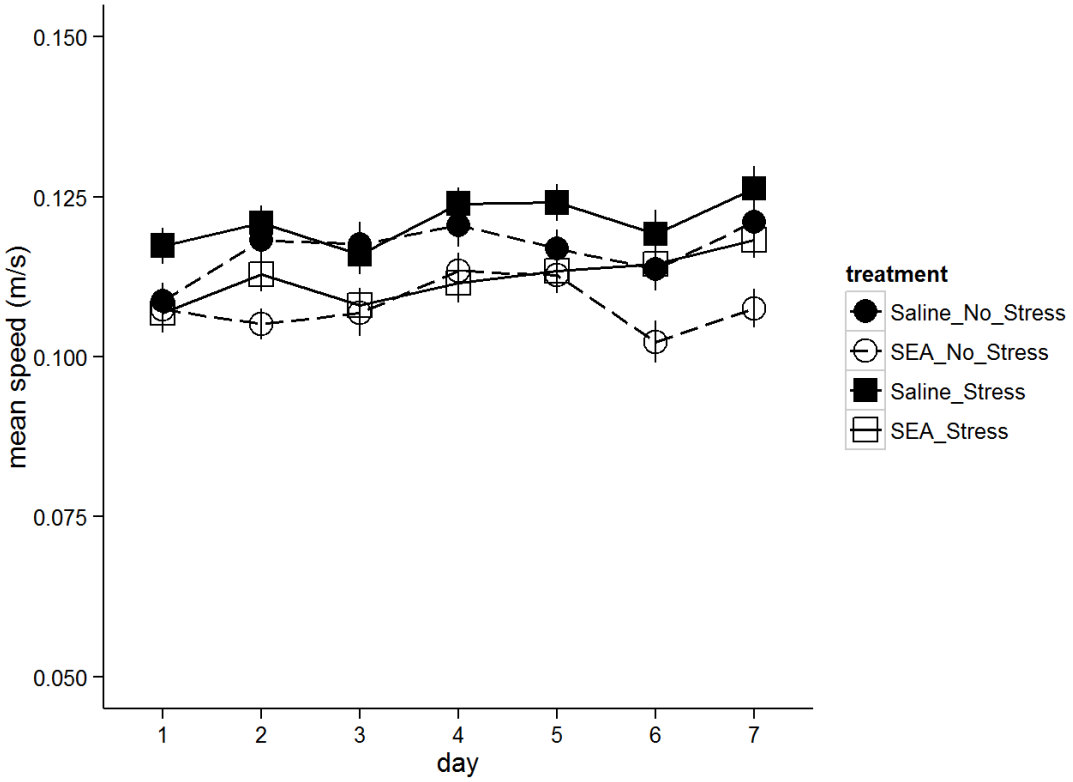

Figure 8. Swim speed in the water radial arm maze. Data expressed as mean speed per treatment per day +/- standard error of the mean (SEM). Speed did not appear to differ between treatment groups ($F(3, 56) = 1.42$, p=n.s), across days ($F(1, 56) = 0.41$, p=n.s), or by sex ($F(1, 56) = 0.86$, p=n.s).

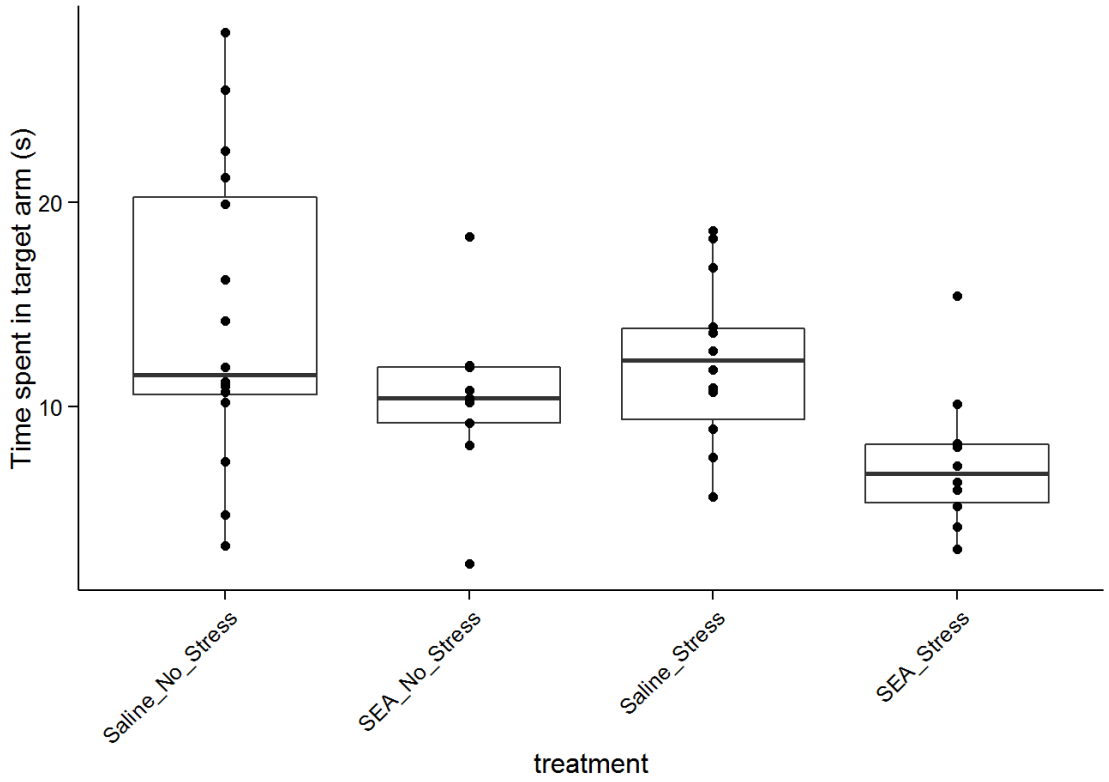

Figure 9. Time spent in the target (platform) arm during probe trial. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Significant main effect of treatment ($F(3, 44) = 3.64$, $p < 0.05$). Pairwise comparison with Tukey's HSD. SEA-Stress animals spent less time in the target arm compared to Saline-No Stress control animals.

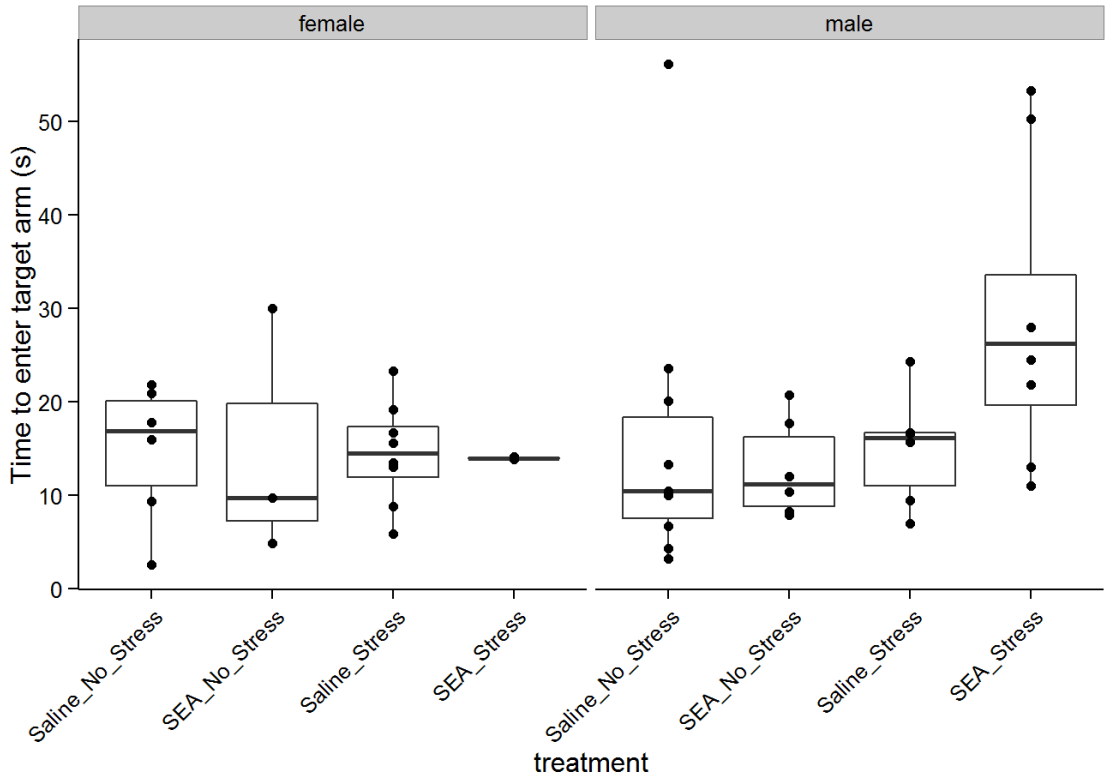

Figure 10. Time to enter the target (platform) arm during probe trial, faceted by sex. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Data split into females and males. Significant three-way interaction of SEA by stress by sex (b = 0.91, Z = 2.7, p < 0.01), with male SEA-Stress animals taking longer to enter the target arm for the first time.

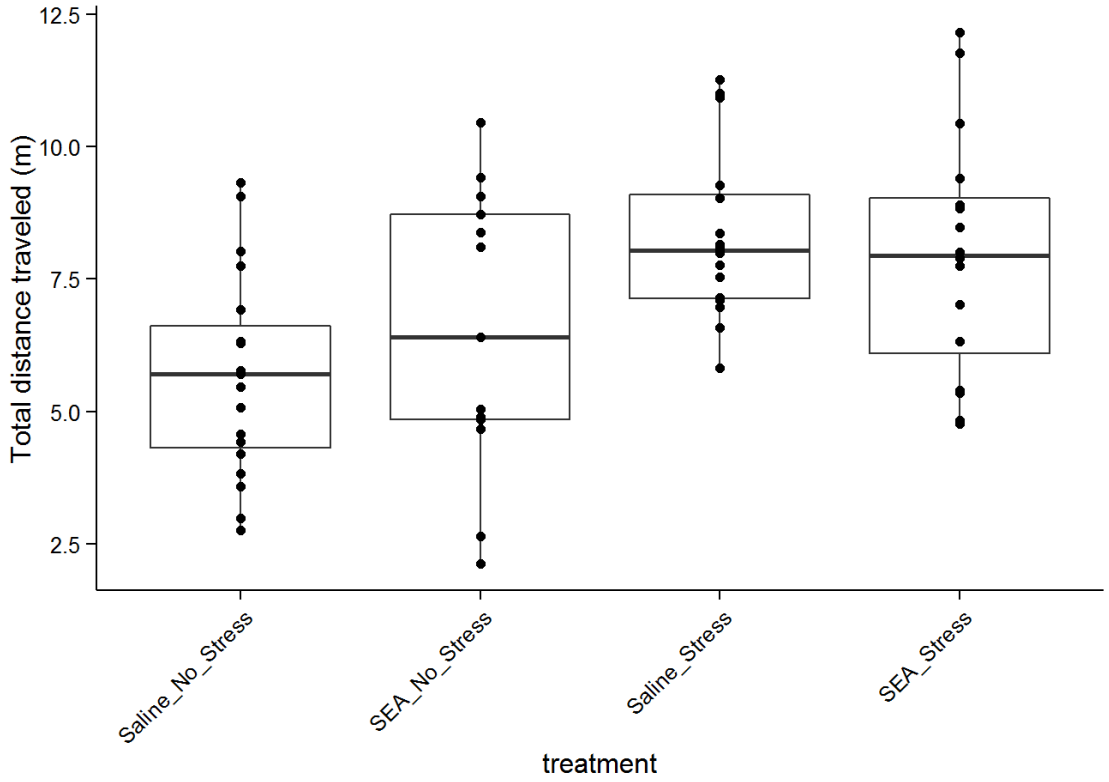

Figure 11. Distance traveled in the elevated plus maze Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Significant main effect of stress (b = 2.6, F(1, 59) = 15.35, p < 0.05), with stress treatment animals moving more distance.

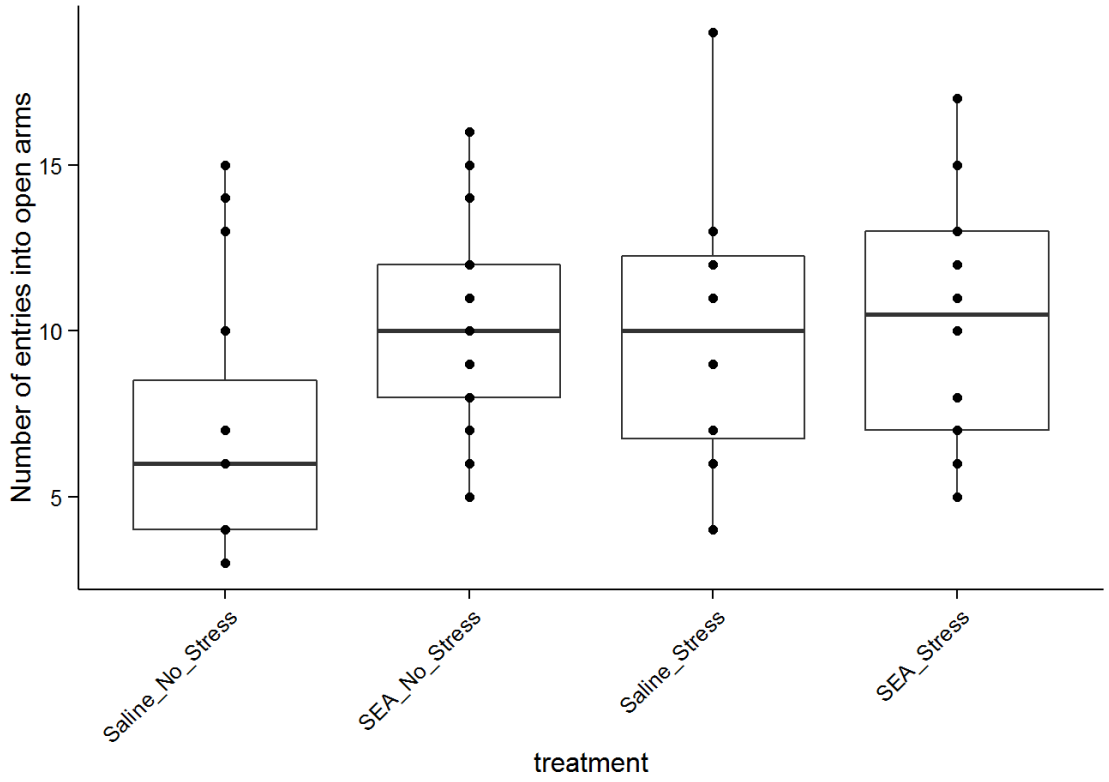

Figure 12. Number of entries into open arms in the elevated plus maze. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Neither an effect of maternal SEA ($F(1, 59) = 2.95$, p=n.s) nor stress ($F(1, 59) = 2.70$, p=n.s) was detected. Similarly an effect of sex was not detected ($F(1, 59) = 3.80$, p=n.s).

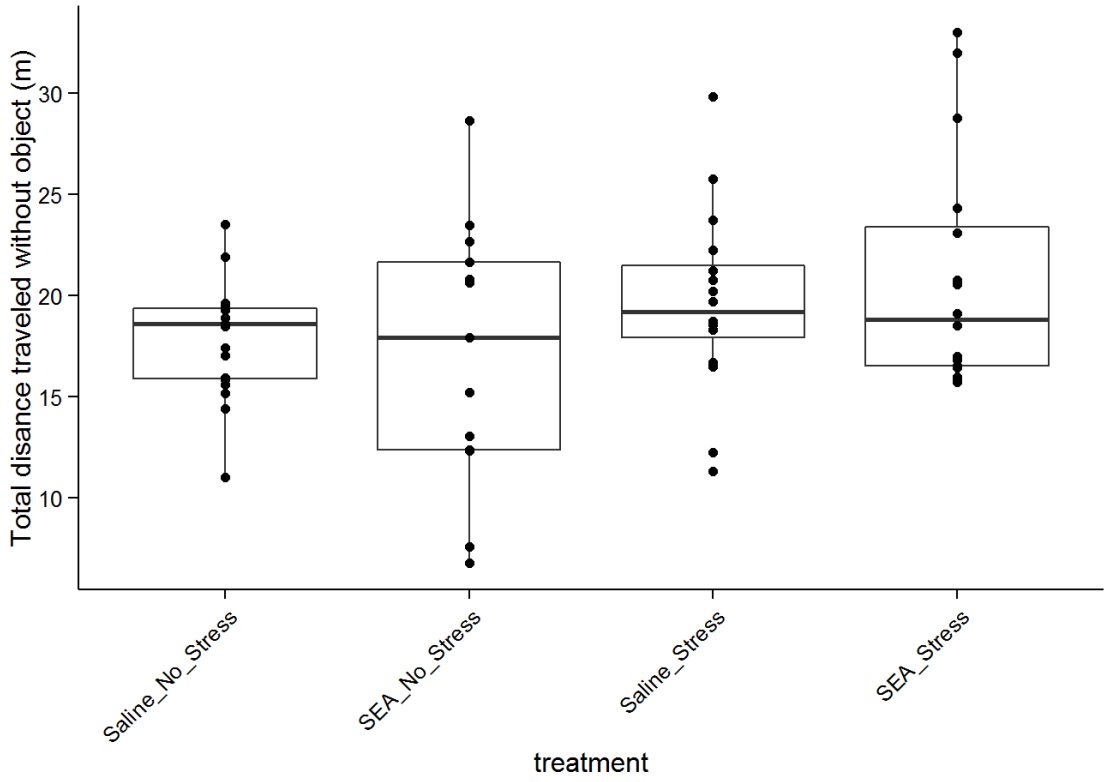

Figure 13. Distance traveled in the open field without novel object. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Significant main effect of stress (b = 2.67, F(1, 61) = 4.62, p < 0.05), with stress treatment animals moving more distance.

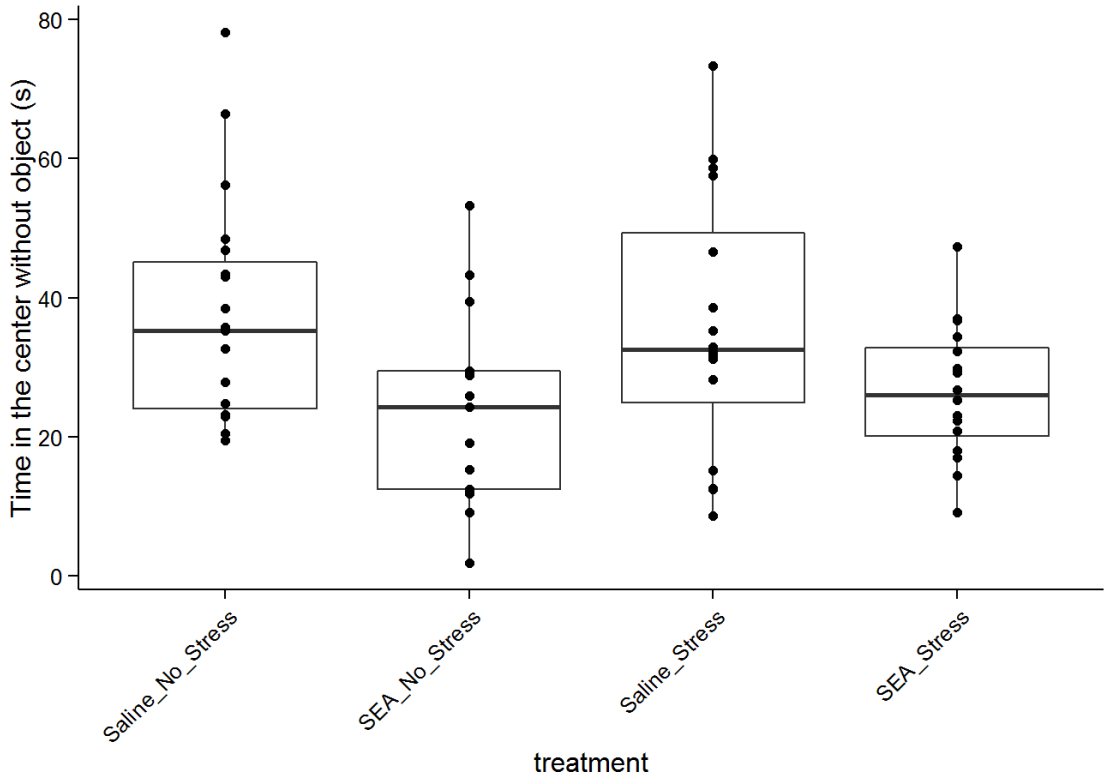

Figure 14. Time spent in the open, center area of the open field without novel object. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. Significant main effect of maternal SEA treatment (b = -11.93, $F(1, 61) = 8.60$, $p < 0.05$), with SEA treatment animals spending less time in the center area.

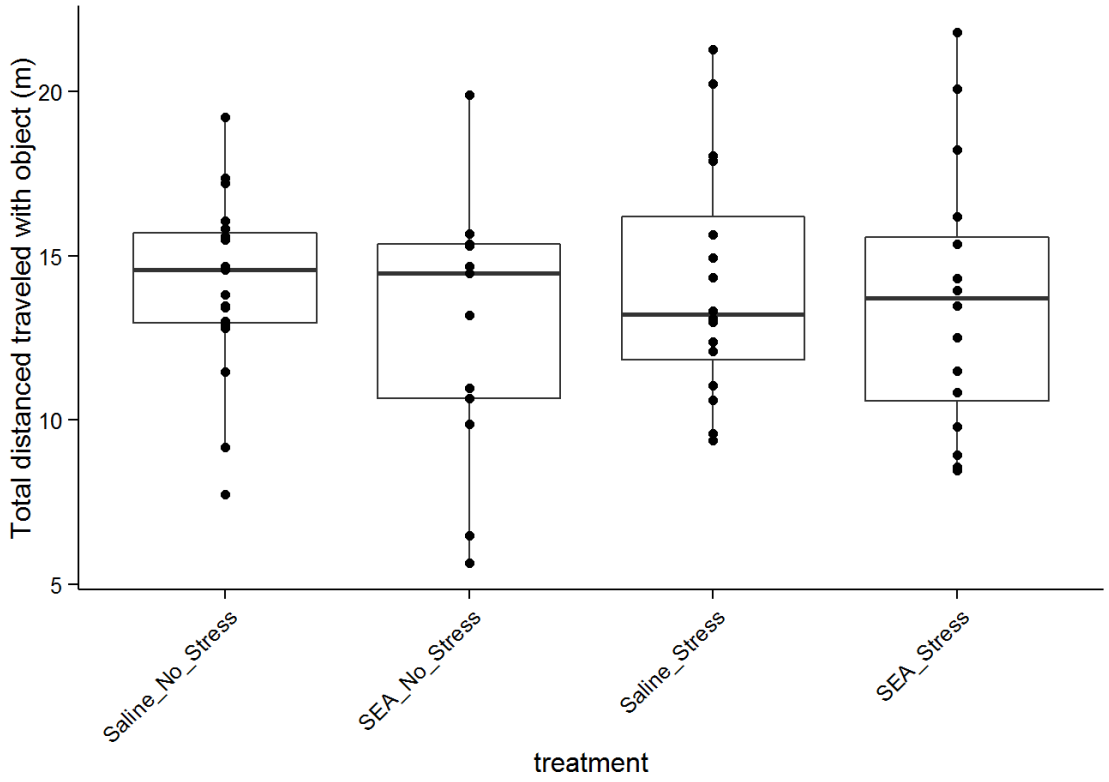

Figure 15. Distance traveled in the open field with a novel object. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. There were no statistically significant effects of either maternal SEA treatment ($F(1, 61) = 0.96$, p=n.s) or stress treatment ($F(1, 61) = 0.13$, p=n.s).

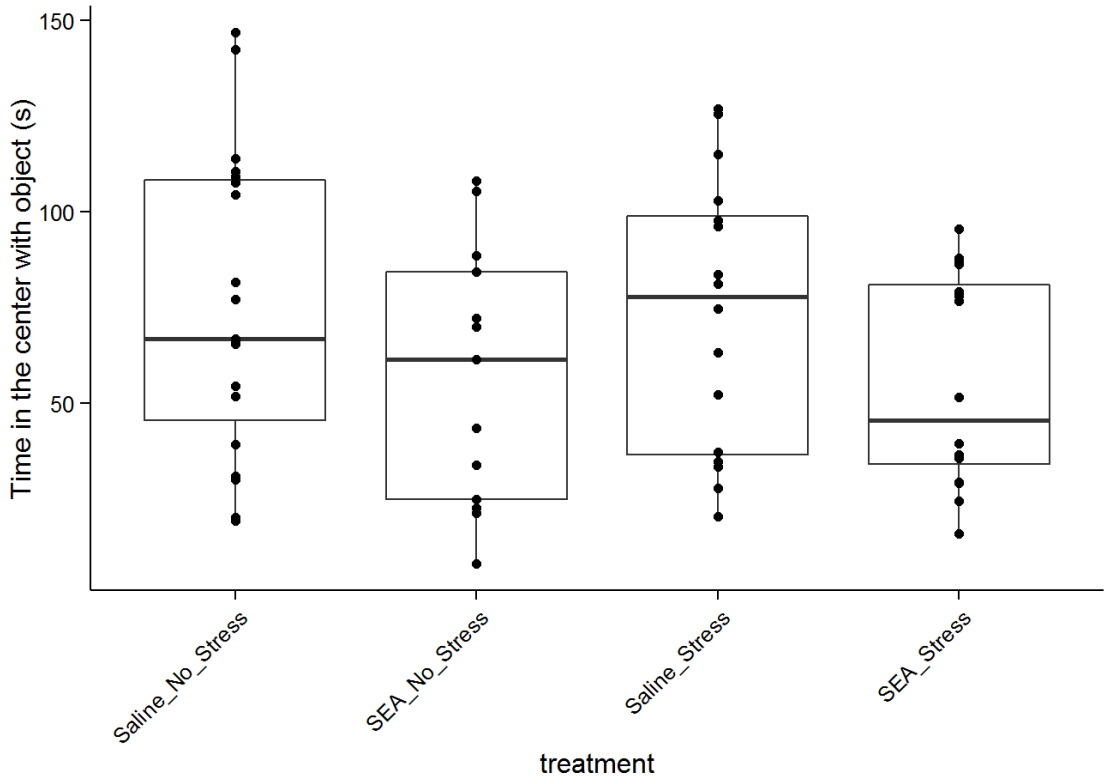

Figure 16. Time spent in the open, center area of the open field with a novel object. Data expressed as individual data points and box plots, with horizontal bold lines representing treatment means. There was a significant main effect of maternal SEA treatment (*b* = -18.07, F(1, 61) = 4.44, *p* <0.05), with SEA treatment animals spending less time in the center area.

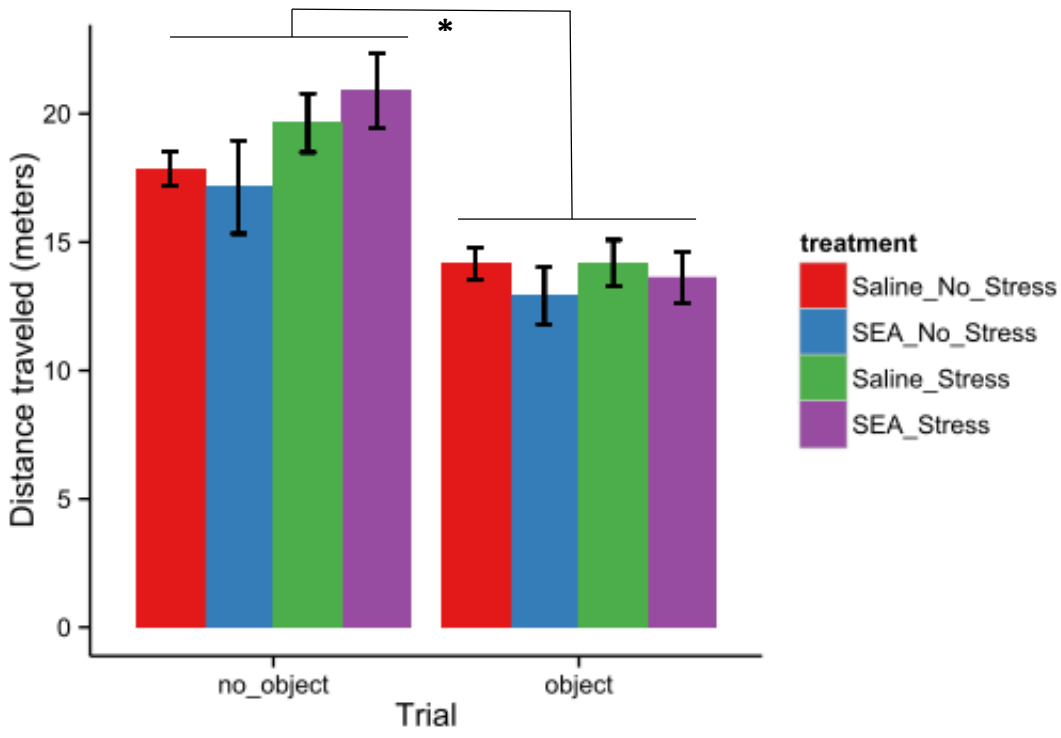

Figure 17. Distance traveled in the open field with and without a novel object. Data expressed as means, +/- the standard error of the mean (SEM). Significant main effect of trial ($F(1, 63) = 86.88$, $p < 0.05$), with all animals moving less distance in the object trial.

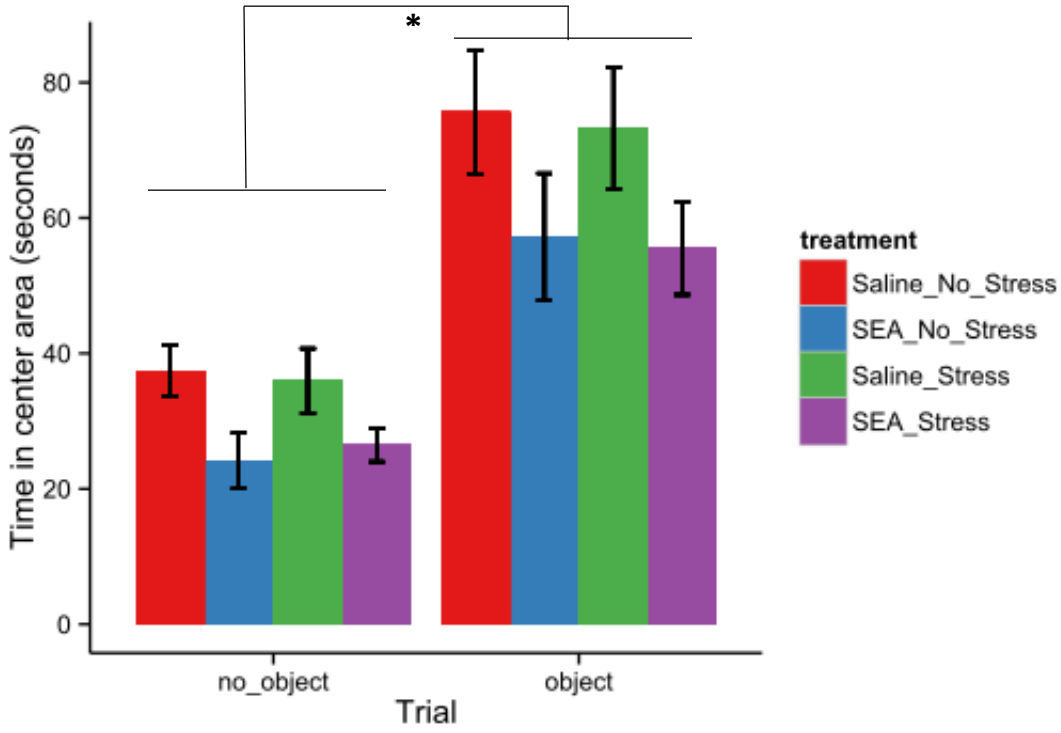

Figure 18. Time spent in the open, center area of the open field with a novel object. Data expressed as means, +/- the standard error of the mean (SEM). Significant main effect of trial ($F(1, 63) = 77.48$, $p < 0.05$), with all animals spending more time in the center area during the object trial.

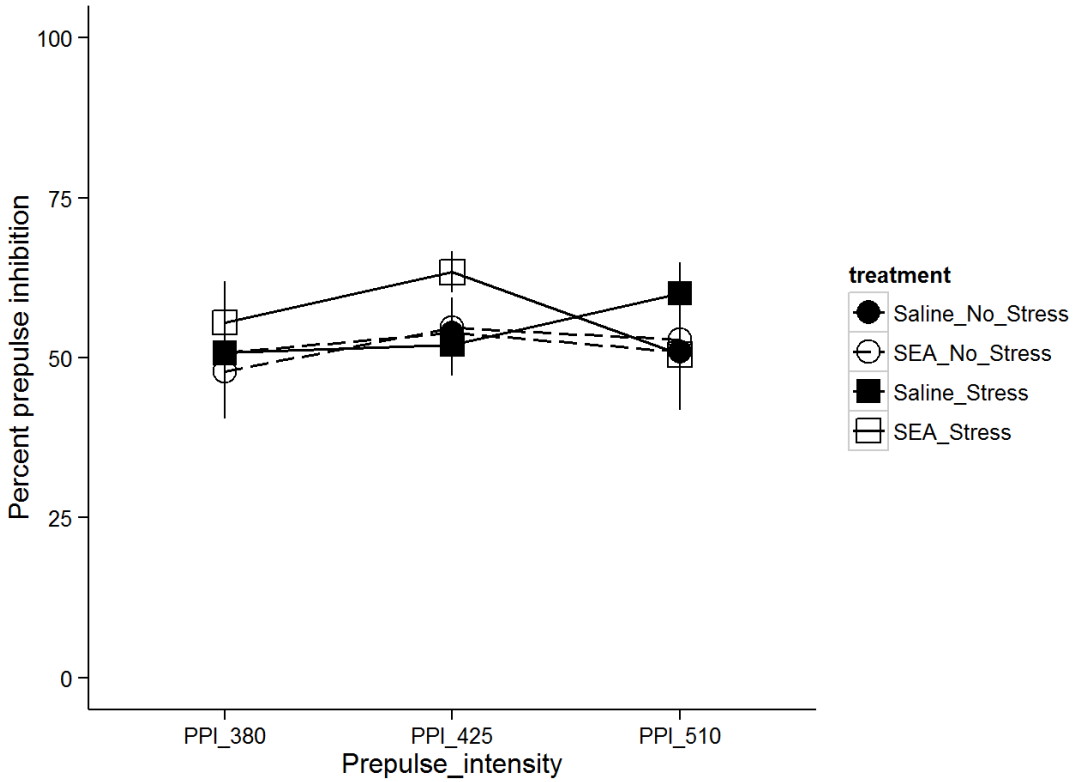

Figure 19. Average percent prepulse inhibition. Data expressed as means, +/- the standard error of the mean (SEM). No significant differences detected for either prepulse intensity ($F(2, 120) = 1.69$, p=n.s) or treatment ($F(3, 59) = 0.20$, p=n.s)

H. References

Adiko, A. C., Babdor, J., Gutierrez-Martinez, E., Guermonprez, P., & Saveanu, L. (2015). Intracellular Transport Routes for MHC I and Their Relevance for Antigen Cross-Presentation. *Front Immunol, 6*, 335. doi: 10.3389/fimmu.2015.00335

Akaike, M., Ohno, H., Tsutsumi, S., & Omosu, M. (1994). Comparison of four spatial maze learning tests with methylnitrosourea-induced microcephaly rats. *Teratology, 49*(2), 83-89. doi: 10.1002/tera.1420490204

Appiah, G. D., Blanton, L., D'Mello, T., Kniss, K., Smith, S., Mustaquim, D., . . . Prevention. (2015). Influenza activity - United States, 2014-15 season and composition of the 2015-16 influenza vaccine. *MMWR Morb Mortal Wkly Rep, 64*(21), 583-590.

Babri, S., Doosti, M. H., & Salari, A. A. (2014). Strain-dependent effects of prenatal maternal immune activation on anxiety- and depression-like behaviors in offspring. *Brain Behav Immun, 37*, 164-176. doi: 10.1016/j.bbi.2013.12.003

Bilbo, S. D., Rudy, J.W., Watkins, L.R., Maier, S.E. (2006). A behavioral characterization of neonatal infection-facilitated memory impairment in adult rats. *Behavioural Brain Research*(169), 8. doi: 10.1016/j.bbr.2005.12.002

Boersma, G. J., Treesukosol, Y., Cordner, Z. A., Kastelein, A., Choi, P., Moran, T. H., & Tamashiro, K. L. (2015). Exposure to activity-based anorexia impairs contextual learning in weight-restored rats without affecting spatial learning, taste, anxiety, or dietary-fat preference. *Int J Eat Disord*. doi: 10.1002/eat.22489

Boksa, P. (2010). Effects of prenatal infection on brain development and behavior: a review of findings from animal models. *Brain Behav Immun, 24*(6), 881-897. doi: 10.1016/j.bbi.2010.03.005

Braff, D. L., & Geyer, M. A. (1990). Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry, 47*(2), 181-188.

Deng, X. Y., Xue, J. S., Li, H. Y., Ma, Z. Q., Fu, Q., Qu, R., & Ma, S. P. (2015). Geraniol produces antidepressant-like effects in a chronic unpredictable mild stress mice model. *Physiol Behav, 152*(Pt A), 264-271. doi: 10.1016/j.physbeh.2015.10.008

Deverman, B. E., & Patterson, P. H. (2009). Cytokines and CNS development. *Neuron, 64*(1), 61-78. doi: 10.1016/j.neuron.2009.09.002

Eaves, C. J. (2015). Hematopoietic stem cells: concepts, definitions, and the new reality. *Blood, 125*(17), 2605-2613. doi: 10.1182/blood-2014-12-570200

Enayati, M., Solati, J., Hosseini, M. H., Shahi, H. R., Saki, G., & Salari, A. A. (2012). Maternal infection during late pregnancy increases anxiety- and depression-like behaviors with increasing age in male offspring. *Brain Res Bull, 87*(2-3), 295-302. doi: 10.1016/j.brainresbull.2011.08.015

Erburu, M., Munoz-Cobo, I., Dominguez-Andres, J., Beltran, E., Suzuki, T., Mai, A., . . . Tordera, R. M. (2015). Chronic stress and antidepressant induced changes in Hdac5 and Sirt2 affect synaptic plasticity. *Eur Neuropsychopharmacol*. doi: 10.1016/j.euroneuro.2015.08.016

Feigenson, K. A., Kusnecov, A. W., & Silverstein, S. M. (2014). Inflammation and the two-hit hypothesis of schizophrenia. *Neurosci Biobehav Rev, 38*, 72-93. doi: 10.1016/j.neubiorev.2013.11.006

Giovanoli, S., Engler, H., Engler, A., Richetto, J., Voget, M., Willi, R., . . . Meyer, U. (2013). Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. *Science, 339*(6123), 1095-1099. doi: 10.1126/science.1228261

Gur, R. C., & Gur, R. E. (2013). Memory in health and in schizophrenia. *Dialogues Clin Neurosci, 15*(4), 399-410.

Hsiao, E. Y., & Patterson, P. H. (2012). Placental regulation of maternal-fetal interactions and brain development. *Dev Neurobiol, 72*(10), 1317-1326. doi: 10.1002/dneu.22045

Jaaskelainen, E., Haapea, M., Rautio, N., Juola, P., Penttila, M., Nordstrom, T., . . . Miettunen, J. (2015). Twenty Years of Schizophrenia Research in the Northern Finland Birth Cohort 1966: A Systematic Review. *Schizophr Res Treatment, 2015*, 524875. doi: 10.1155/2015/524875

Jameson, S. C., & Bevan, M. J. (1998). T-cell selection. *Curr Opin Immunol, 10*(2), 214-219.

Kaufman, G. N., Massoud, A. H., Dembele, M., Yona, M., Piccirillo, C. A., & Mazer, B. D. (2015). Induction of Regulatory T Cells by Intravenous Immunoglobulin: A Bridge between Adaptive and Innate Immunity. *Front Immunol, 6*, 469. doi: 10.3389/fimmu.2015.00469

Klug, M., & van den Buuse, M. (2013). An investigation into "two hit" effects of BDNF deficiency and young-adult cannabinoid receptor stimulation on prepulse inhibition regulation and memory in mice. *Front Behav Neurosci, 7*, 149. doi: 10.3389/fnbeh.2013.00149

Kohl, S., Heekeren, K., Klosterkotter, J., & Kuhn, J. (2013). Prepulse inhibition in psychiatric disorders--apart from schizophrenia. *J Psychiatr Res, 47*(4), 445-452. doi: 10.1016/j.jpsychires.2012.11.018

Kumar, S., & Jack, R. (2006). Origin of monocytes and their differentiation to macrophages and dendritic cells. *J Endotoxin Res, 12*(5), 278-284. doi: 10.1179/096805106X118861

Kumari, V., Soni, W., Mathew, V. M., & Sharma, T. (2000). Prepulse inhibition of the startle response in men with schizophrenia: effects of age of onset of illness, symptoms, and medication. *Arch Gen Psychiatry, 57*(6), 609-614.

Kusnecov, A. W., Liang, R., & Shurin, G. (1999). T-lymphocyte activation increases hypothalamic and amygdaloid expression of CRH mRNA and emotional reactivity to novelty. *J Neurosci, 19*(11), 4533-4543.

Li, X. H., Pang, H. Q., Qin, L., Jin, S., Zeng, X., Bai, Y., & Li, S. W. (2015). HSP70 overexpression may play a protective role in the mouse embryos stimulated by CUMS. *Reprod Biol Endocrinol, 13*, 125. doi: 10.1186/s12958-015-0123-z

Liu, W., Wang, H., Wang, Y., Li, H., & Ji, L. (2015). Metabolic factors-triggered inflammatory response drives antidepressant effects of exercise in CUMS rats. *Psychiatry Res, 228*(3), 257-264. doi: 10.1016/j.psychres.2015.05.102

Makinodan, M., Tatsumi, K., Manabe, T., Yamauchi, T., Makinodan, E., Matsuyoshi, H., . . . Wanaka, A. (2008). Maternal immune activation in mice delays myelination and axonal development in the hippocampus of the offspring. *J Neurosci Res, 86*(10), 2190-2200. doi: 10.1002/jnr.21673

Maynard, T. M., Sikich, L., Lieberman, J. A., & LaMantia, A. S. (2001). Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. *Schizophr Bull, 27*(3), 457-476.

Mednick, S. A., Machon, R. A., Huttunen, M. O., & Bonett, D. (1988). Adult schizophrenia following prenatal exposure to an influenza epidemic. *Arch Gen Psychiatry, 45*(2), 189-192.

Mellman, I., & Steinman, R. M. (2001). Dendritic cells: specialized and regulated antigen processing machines. *Cell, 106*(3), 255-258.

Meyer, U., Feldon, J., Schedlowski, M., & Yee, B. K. (2006). Immunological stress at the maternal-foetal interface: a link between neurodevelopment and adult psychopathology. *Brain Behav Immun, 20*(4), 378-388. doi: 10.1016/j.bbi.2005.11.003

Meyer, U., Nyffeler, M., Schwendener, S., Knuesel, I., Yee, B. K., & Feldon, J. (2008). Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical

dysfunction after in utero immune challenge. *Neuropsychopharmacology, 33*(2), 441-456. doi: 10.1038/sj.npp.1301413

Mortensen, P. B., Pedersen, C. B., Westergaard, T., Wohlfahrt, J., Ewald, H., Mors, O., . . . Melbye, M. (1999). Effects of family history and place and season of birth on the risk of schizophrenia. *N Engl J Med, 340*(8), 603-608. doi: 10.1056/NEJM199902253400803

Muller, N., Weidinger, E., Leitner, B., & Schwarz, M. J. (2015). The role of inflammation in schizophrenia. *Front Neurosci, 9*, 372. doi: 10.3389/fnins.2015.00372

Proft, T., & Fraser, J. D. (2003). Bacterial superantigens. *Clin Exp Immunol, 133*(3), 299-306.

Richetto, J., Calabrese, F., Meyer, U., & Riva, M. A. (2013). Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. *Brain Behav Immun, 33*, 190-200. doi: 10.1016/j.bbi.2013.07.006

Soumiya, H., Fukumitsu, H., & Furukawa, S. (2011). Prenatal immune challenge compromises development of upper-layer but not deeper-layer neurons of the mouse cerebral cortex. *J Neurosci Res, 89*(9), 1342-1350. doi: 10.1002/jnr.22636

Sun, J., & Braciale, T. J. (2013). Role of T cell immunity in recovery from influenza virus infection. *Curr Opin Virol, 3*(4), 425-429. doi: 10.1016/j.coviro.2013.05.001

Tajima-Pozo, K., de Castro Oller, M. J., Lewczuk, A., & Montanes-Rada, F. (2015). Understanding the direct and indirect costs of patients with schizophrenia. *F1000Res, 4*, 182. doi: 10.12688/f1000research.6699.2

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols, 1*(2), 848-858. doi: 10.1038/nprot.2006.116

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols, 2*(2), 322-328. doi: DOI 10.1038/nprot.2007.44

Walker, A. K., Nakamura, T., & Hodgson, D. M. (2010). Neonatal lipopolysaccharide exposure alters central cytokine responses to stress in adulthood in Wistar rats. *Stress, 13*(6), 506-515. doi: 10.3109/10253890.2010.489977

Xu, X., Zhang, S., Li, P., Lu, J., Xuan, Q., & Ge, Q. (2013). Maturation and emigration of single-positive thymocytes. *Clin Dev Immunol, 2013*, 282870. doi: 10.1155/2013/282870

Zhang, Z., & van Praag, H. (2015). Maternal immune activation differentially impacts mature and adult-born hippocampal neurons in male mice. *Brain Behav Immun, 45*, 60-70. doi: 10.1016/j.bbi.2014.10.010