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Effects of Maternal Immune Activation on the Outcome of Traumatic Brain Injury

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Alan James To

Committee in Charge:

Neil Richtand, Chair
Byungkook Lim, Co-Chair
Aaron Coleman

2019

The Thesis of Alan James To is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California San Diego

2019

Dedication

This work is dedicated to my parents, Christina and Michael To

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ABSTRACT OF THE THESIS

Effects of Maternal Immune Activation on the Outcome of Traumatic Brain Injury

by

Alan James To

Master of Science in Biology

University of California San Diego, 2019

Professor Neil Richtand, Chair
Professor Byungkook Lim, Co-Chair

Studies have shown that inflammation is the main mediator of Traumatic Brain injury (TBI). Activation of a pregnant mother's immune system, termed Maternal Immune Activation (MIA), has a long-standing effect upon inflammatory response in adolescent and adult offspring. For the first time, we hypothesized a synergistic effect between MIA and TBI, due to the influence of MIA upon central inflammatory response. Pregnant mice were injected with Polyinosinic:Polycytidylic Acid (Poly I:C) to induce

MIA. TBI was induced using Controlled Cortical Impact (CCI). A series of behavioral assays were performed to assess severity of, and recovery from, TBI in control and MIA groups: Inverted Grid, Balance Beam, Open Field, Prepulse Inhibition of Startle, and Fear Conditioning. Our data identified effects of surgery on all behavioral assays performed. It also identified significant effects of MIA treatment in Inverted grid, Balance Beam, PPI, and Fear Conditioning. However, the MIA treatment effect was only found in sham animals for inverted grid and PPI. In Balance beam, MIA treatment was found only in TBI animals. Lastly for fear conditioning, a surgery effect was found only in MIA animals. Our study identified effects of MIA on both inverted grid and balance beam behavioral outcome measures during recovery from TBI. Effects of MIA upon fear conditioning were also observed following TBI. In combination, these data represent the first demonstration of impact of MIA on recovery following TBI. Further investigation is needed to determine clinical and epidemiological consequences of these effects in human populations.

Introduction

Traumatic Brain Injury (TBI) is an injury to the brain that causes a cascade of damages, which can alter speech, vision, motor functions, memory, mood, and normal behaviors. In 2015, a statistical analysis from the Brain Injury Association of America reported more than 2.5 million new brain injury cases each year. Survivors of a TBI may suffer chronic neurological and behavioral deficits (McAllister 2008). It was estimated that medical and rehabilitation costs of TBI are $300 million annually. This makes TBI one of the most costly and treatment-heavy injuries.

With current war situations in Iraq and Afghanistan, TBI has been described as the signature injury in military personnel (Arriola and Rozelle 2016; Risdall and Menon 2011; Taylor et al. 2012). The source of injury can be from a direct impact to the head or a blast injury from explosion (Warden 2006). Dependent on the severity of the injury military personnel acquire, they may suffer from long-term neurological disorders (Arriola and Rozelle 2016). Additionally, it was shown that a mild TBI increases the risk for developing post-traumatic stress disorder (PTSD), drug and alcohol abuse, depression, and behavior change (Bryant 2011; Elliott et al. 2015; Isaac et al. 2015; Morissette et al. 2011; Yurgil et al. 2014). Recent publications have shown that there is an increase of survivors from war due to improvement in protection (Eve et al. 2016). In 2009 after deployment, over 60,000 veterans were found to have symptoms of TBI contributing to over $150 billion in healthcare costs (Arriola and Rozelle 2016).

There are multiple levels defining the severity of the brain injury; mild, moderate, and severe (Fluiter et al. 2014; Niyonkuru et al. 2013; Yu et al. 2009). The Glasgow Coma Scale (GCS) is a 15-point rating system that determines severity based on vision,

speech, and motor responses. Mild TBI is the least severe form of injury and is commonly associated with amnesia that may last up to 24 hours and a GCS score of 13-15. Moderate TBI is associated with amnesia that may extend up to a week and a GCS score of 9-12. Lastly, severe TBI is associated with amnesia that may last longer than a week and a GCS score of 8 and under (Bryant 2011; Carroll et al. 2004; Eve et al. 2016; Ruff et al. 2009; Sternbach 2000; Teasdale et al. 2014). The severity of TBI resulting from our surgery, the impact made from a 1.5mm depth CCI, is comparable to severe TBI (Romine et al. 2014; Yu et al. 2009). Dependent on the severity level, TBI may lead to permanent damage (Gyoneva and Ransohoff 2015). TBI may cause issues that impact the way a person thinks, behaves, and lives. Some common symptoms include headaches, irritability, depression, difficulty with concentration, and vertigo. The determining factor of these symptoms is dependent on both severity and site of the impact (Siopi et al. 2012). The site of impact is very important, as different areas of the brain control different motor and cognitive functions. Two patients of Pierre Broca sustained an injury to the inferior frontal gyrus. An injury to this area, later named as Broca's area, resulted in impairment to speech production (Dronkers et al. 2007). Injury to Wernicke's area, located in the superior temporal gyrus (STG), will result in impairment to connect words into meaningful sentences (DeWitt and Rauschecker 2013).

TBI can be separated into two time-dependent phases; primary and secondary. In the primary phase, the initial impact may cause physical injury such as destruction of blood vessels and neuronal cell death. A cascade of secondary events follow that

consists of inflammation, excitotoxicity, mitochondrial dysfunction, oxidative stress, and disruption of the Blood-Brain Barrier (BBB) (Chodobski et al. 2011; Hiebert et al. 2015; Lozano et al. 2015; Quillinan et al. 2016; Woodcock and Morganti-Kossmann 2013). Glutamate, as the major excitatory neurotransmitter, activates ionotropic and metabotropic glutamate receptors that normally mediate synaptic transmission including N-methyl-D-asparate (NMDA), AMPA, and mGluR1 receptors (Dong et al. 2009; Meldrum 2000). Previous studies have found increased levels of extracellular glutamate upon a TBI (Globus et al. 1995; Koizumi et al. 1997; Obrenovitch and Urenjak 1997). Following TBI, glutamate accumulates/increases (Nilsson et al. 1990) and glutamate receptors become hyperactivated in a non-controllable way leading to cell death (Mehta et al. 2013; Salinska et al. 2005). A study showed administration of a non-competitive NMDA glutamate receptor antagonist after injury prevents neuronal damage, which implies that glutamate, through NMDA receptor activation, plays a critical role in TBI (Rao et al. 2001). When NMDA receptors are overactivated, an excessive influx of calcium initiates the production of reactive oxygen species, which is harmful to the mitochondria (Mehta et al. 2013). Hence, overactivation of these permeable channels is an important cause of long-term damage in TBI, and may contribute to other neurodegenerative diseases (Doble 1999; Dong et al. 2009; Hynd et al. 2004; Ong et al. 2013).

The mitochondria are the powerhouses of the cell creating ATP from glycolysis, Krebs cycle, and the electron transport chain (ETC). Excessive calcium uptake, a consequence of excitotoxity, commonly results in oxidative stress, in turn creating

reactive oxygen species (ROS) (Mehta et al. 2013). Overproduction of ROS produces free radicals that are harmful to cells (Hiebert et al. 2015; Mutinati et al. 2014). In normal functioning of the mitochondria, ROS are inactivated by production of endogenous antioxidants through the mitochondrial permeability transition pore (mPTP). Abundance of ROS will offset the available mPTP and ultimately cause a burst of ROS that destroys the mitochondria (Hiebert et al. 2015). Upon a TBI, mitochondrial-specific apoptotic effectors are also released to the cytosol, which bind to inhibitor of apoptosis proteins and allow for apoptosis to occur. This process is a condition called mitochondrial dysfunction (Hiebert et al. 2015), which ultimately kills the cell.

The blood-brain barrier serves as a protective layer that protects the brain and central nervous system from the external environment, including the bloodstream (Abbott and Friedman 2012). In the event of an injury, this permeability barrier may be compromised allowing proinflammatory cells in the periphery to infiltrate the brain resulting in edema (Boyd et al. 2015; Chodobski et al. 2011; Jungner et al. 2016; Lifshitz et al. 2004). In our experiment, TBI and sham surgeries were performed. Sham animals receive a craniotomy, whereas TBI animals receive a craniotomy plus a physical traumatic impact to the brain. It is important to note that the BBB is not disrupted in the Sham animals as a craniotomy is made with an intact dura mater; however, the BBB is disrupted in the TBI surgery animals.

The main mediator of the secondary injuries in TBI is inflammation, as interactions between these mechanisms described above mediate the pathophysiology of TBI (Woodcock and Morganti-Kossmann 2013). When an injury occurs, chemokines,

a immune cell recruiter, recruits pro-inflammatory cytokines such as IL-1, TNF-α, IL-6, and interferon-γ to the site of the injury (Lozano et al. 2015; Woodcock and Morganti-Kossmann 2013). IL-6 in particular, is directly linked to the severity of injury (Yang et al. 2013a). Immune cells in the brain such as microglial cells and astrocytes are also upregulated upon a brain injury, producing more cytokines. Production of these inflammatory cytokines centrally and peripherally disrupt the physical interaction between the BBB endothelial cells and astrocytic glial cells compromising the permeability of the BBB allowing peripheral immune cells to enter the brain (Das et al. 2012; Lozano et al. 2015; Utagawa et al. 2008; Woodcock and Morganti-Kossmann 2013). Working alongside of these pro-inflammatory cytokines, anti-inflammatory cytokines counteract the effects of inflammation. A well-studied anti-inflammatory cytokine, IL-10, is said to inhibit production of pro-inflammatory cytokines (Woodcock and Morganti-Kossmann 2013).

The cascade of these complex molecular mechanisms will all lead to inflammation, cell death, and neurodegradation (Corps et al. 2015). It causes the injury to persist or worsen, and is a major determinant of the level of behavioral impairment (Lozano et al. 2015; Yu et al. 2009). Published literature has found that patients who experienced a mild form of TBI may still experience long-term cognitive dysfunction (Corrigan et al. 2004; Pagulayan et al. 2006). Thus, the interplay between TBI mechanisms is complex and can induce life long neurological and neuropsychological deficiencies (Quillinan et al. 2016).

1.1 Maternal Immune Activation

Maternal immune activation is an innate immune response in a pregnant mother produced by an environmental factor; such as influenza, bacterial, or viral infection (Patterson 2009; Vorhees et al. 2015). In our experiment, a viral analogue, Polyinosinic:Polycytidylic acid (Poly I:C) will be administered to pregnant mice at gestation day 12. Poly I:C is a synthetic double-stranded (ds) RNA, which mimics a viral infection due to the fact that viruses produce many dsRNA during replication. Toll-like receptor 3 (TLR3) recognizes agents, such as Poly I:C, through PAMPs (pathogen associated molecular patterns) and activates Nuclear Factor (NF)-κB (Alexopoulou et al. 2001; Fortier et al. 2004; Kumar et al. 2006; Reisinger et al. 2015). This further stimulates interferons (IFNs) and cytokines, which include IL-6, IL-8, and IL-12. *In utero* exposure to these inflammatory cytokines results in longstanding cellular and behavioral alterations in the adult MIA offspring (Richtand et al. 2012b; Vorhees et al. 2015; Zuckerman et al. 2003).

When the maternal immune system is activated during gestation, inflammatory cytokines pass through the placenta to the offspring (Hsiao and Patterson 2012). Such cytokines include IL-1, IL-6, TNF-α, and IL-10 (Beloosesky et al. 2010; Hodyl et al. 2008; Hodyl et al. 2007). Previous publications have found that after Poly I:C injection there is an upregulation of IL-6, TNF-α, and IL-1 that lasts up to 24 hours in the placenta, a site of maternal-fetal interaction (Hsiao and Patterson 2011). This transfer of cytokines has been shown to cause long-standing alterations in offspring's central (Arrode-Bruses and Bruses 2012; Garay et al. 2013) and peripheral inflammatory

response into adulthood (Meyer et al. 2005; Missault et al. 2014; Onore et al. 2014; Ozawa et al. 2006; Patrich et al. 2016; Patterson 2002). These effects have been studied in the Maternal Immune Activation (MIA) animal model (Brown and Patterson 2011).

In particular, IL-6 was shown sufficient to cause maternal immune activation after a single injection and is able to produce behavioral deficits similar to the MIA model (Patterson et al. 2008; Smith et al. 2007). This was supported through co-administration of anti-IL-6 antibody to MIA animals, extinguishing behavioral alterations associated with MIA (Smith et al. 2007). Lastly, IL-6 knockout mice with MIA, results in offspring only exhibiting some of the behaviors normally seen in wild-type MIA (Smith et al. 2007). Furthermore, adult offspring of pregnant mice injected with IL-6 or poly I:C both showed a deficit in prepulse inhibition (PPI) of startle (Smith et al. 2007). Prepulse Inhibition is a normal suppression of the startle response from a startling stimulus when a barely detectable stimulus precedes it (Braff et al. 2001; Graham 1975). The phenomenon in which there was a deficit in PPI was found in people with schizophrenia, which interestingly is also seen in first-degree family members (Cadenhead 2011; Hasenkamp et al. 2010). In addition, publications have seen behaviors of relevance to schizophrenia in MIA offspring with one being deficits in PPI of startle (Meyer et al. 2006b; Shi et al. 2003; Wolff and Bilkey 2010). Due to the similarities seen in both humans and animal models, PPI became a great way to study sensorimotor gating deficits (Braff et al. 2001; Geyer et al. 2001).

Many publications have looked into TBI and MIA as independent studies, however we are not aware of prior studies examining the impact of MIA on outcome following TBI. Since inflammatory signaling, mediated by cytokine response, is a key determinant of TBI outcome, and inflammatory response is altered in MIA offspring, it is to our interest to determine if MIA affects outcome measures of TBI. Therefore, to test our hypothesis, we explore the effects of MIA upon a group of motor and non-motor outcomes after experimental TBI. A detailed timeline of the experimental design is shown in Fig. 1. The MIA models will first be bred using a double stranded RNA viral analogue, Polyinosinic-Polycytidylic (Poly I:C), to induce a maternal immune response. TBI will be induced using the method of Controlled Cortical Impact. The main areas in which the injury may affect, but are not limited to, include the motor cortex, auditory cortex, visual cortex, and the hippocampus. The areas of injury are detailed in Fig. 2 and Table 1.

The cerebral cortex can be divided into several distinct areas. The motor cortex regulates planning, controlling, and executing movements (Cheney 1985; Scott 2003). The auditory cortex regulates hearing (Warrier et al. 2009) and the visual cortex processes visual stimuli (Lee et al. 1998). The somatosensory cortex regulates the sensation of touch in different areas of the body (Abraira and Ginty 2013). The main functions of the hippocampus are long-term memory consolidation and spatial navigation (Burgess et al. 2002). Damage to this area will result in impairment of forming new memories while old memories are preserved (Bird and Burgess 2008).

To measure dimensions of overall function following damage to the cortex, animals of different treatments and surgery will be subjected to a motor battery. Motor strength will be assessed through an Inverted Grid Test. Motor coordination and balance will be assessed through a Balance Beam Test. Locomotion will be assessed through Open Field. Sensorimotor gating will be assessed through Prepulse Inhibition of Startle. PPI will be used in this study because MIA offspring have been found to exhibit a deficit in this behavioral measure (Meyer et al. 2006b; Shi et al. 2003; Wolff and Bilkey 2010) and it is of interest to investigate the outcome following a TBI. Lastly, anxiety and fear conditioned responding will be assessed through fear conditioning. These behavioral experiments serve to determine the effects of MIA across different dimensional outcomes following TBI. The broader goals of the study are to give insight into whether MIA impacts recovery from TBI, as potential effects upon health outcome could alter one's behavior and way of life.

Materials and Methods

2.1 Animals

All animal procedures were approved by the Veterans Affairs Institutional Animal Care and Use Committee (San Diego, California). All animals were housed under normal condition with ad libitum access to food and water and a light/dark cycle at 6am/6pm respectively. Male and Female breeders (6-8 weeks old; C57BL/6; Jackson Lab) were used to produce the MIA models. All behavioral experiments were performed after postnatal day 60 (PD60) during the animals' light cycle except for Open Field, which is performed during their dark cycle because that is when the animal is the most active. Baseline values from inverted grid were used to balance randomization of animals to TBI or Sham surgery groups (before PD90). Post surgery, animals' health condition was visually checked daily for 7 days and weekly after until animal is euthanized.

2.2 Maternal Poly I:C Administration

Female breeders were weighed 24 hours before mating. On gestation day 12 (GD12), dams with a weight gain of >5g were identified as pregnant and were randomly given a single intraperitoneal injection of either Poly I:C (20mg/kg) freshly dissolved in saline or saline alone. Dams who gave birth were culled to 6 pups (3 females and 3 males) per litter on PD3. Excess pups from dams with the same treatment were given to dams with fewer than 6 pups. Injected dams that did not give birth were euthanized. Pups were weaned on PD25 and housed up to 4 per cage separated by sex. In order to control for litter effects, a maximum of 2 pups per litter were assigned to each

experimental condition. Offspring of 20 saline treated and 19 Poly I:C treated dams were used in this study.

2.3 Controlled Cortical Impact (CCI)

CCI is a method used to induce a traumatic brain injury with consistent reproducible impact (Hall et al. 2008; Washington et al. 2012). Procedures of CCI were followed as previously described in the literature (Niesman et al. 2014). Mice were anesthetized using 5% vol/vol isoflurane and fixed onto a stereotactic frame. Upon fixation, anesthetization is maintained at 2% and body temperature maintained at 37°C throughout surgery. Eyes were covered with artificial tears to prevent damage during surgery. Buprenorphine (0.1mg/kg) and Cefazolin (15mg/kg) were given subcutaneously and craniotomies were performed using a V35 electric hand piece to the right of bregma. The dimensions of the trapezoidal-shaped craniotomy were: from bregma, 2mm rostral, from there 3mm lateral; from bregma, 3mm caudal, from there 4mm lateral. Final dimensions were 3mm upper width, 4mm lower width, and 5mm length. CCI was induced using a stereotaxic impactor (Impact One; leicabiosystems.com). Utilizing the impactor, a 3mm tip was accelerated perpendicularly to the exposed brain at 5m/second with a depth of 1.5mm and a dwell time of 0.3 seconds. Areas of injury to the brain are illustrated in Fig. 2. Size and location of the craniotomy was estimated base on the site of the injury to which the impactor was used to induce a TBI. The dimensions of direct injury were calculated from Paxinos' Stereostaxic Coordinate (Paxinos and Franklin 2004) using the known length of the

impactor track into the brain and the depth of the impactor descent. These brain regions contribute to motor, auditory, and visual functions. Swelling and bleeding of the brain indicates a good TBI. Scalp was sutured with 7.0 absorbable sutures and was given a topical antibiotic ointment. Animal is removed from the frame and placed in a recovery chamber on a warming blanket. Same procedures were followed for Sham animals except that the impactor tip was lowered but was not allowed to directly touch the brain surface.

2.4 Behavioral Testing

Prior to surgery, a series of motor experiments were performed on mice (2-3 months old) to determine baseline measures. Once baseline is determined, animals were subjected to CCI or sham surgery, and 7 days post surgery, a motor battery followed. 3 weeks after completion of the motor battery, animals were subjected to fear conditioning for four consecutive days and were euthanized upon completion. All experiments in the motor battery were arranged in order from least to most stressful.

2.5 Inverted Grid

Inverted Grid is a motor strength measure that allows detection of neuromuscular deficits (Carlson et al. 2010). A custom made apparatus was used in which a metal wire is fixed 40cm above the base of the table. Soft pads were placed on the table to prevent damage to the animal during their fall. Animals are suspended upside down on the metal wire and their latency to fall was measured. Each animal was subjected to three

trials in which the second trial starts after all animals in the same group completed the first trial. The animal is removed after 30 minutes per trial. The metal wire is cleaned with Air-Rex in between trials.

2.6 Balance Beam

Balance Beam is a task that allows measurements of the animals' balance and motor coordination (Crawley 2007). This experiment was done in sequential order before inverted grid. Animals were habituated to white noise for a minimum of 30 minutes before the start of the experiment. A balance beam apparatus was set up where the animal is on an open platform 50cm above the base of the table. Their task involves walking across an 80cm long x 12mm width acrylic square beam into a 20x20cm cube box. The number of foot slips and the time it took the animal to cross the beam were assessed. Each animal is subjected to three consecutive trials. Beam was cleaned with kimwipes in-between each trial to remove any urine or feces.

2.7 Open Field

Open Field is a measure of the animals' locomotion. Animals were placed in the center of a Plexiglas chamber [16" (W) x 16" (D) x 15" (H)] with white noise playing during the course of the experiment as background noise. A 16x16 Photobeam Activity System (San Diego Instruments, San Diego) that is fixed 1.25cm above the base surrounds the Plexiglas chamber. Behavioral testing was performed during the animal's dark cycle starting at 6pm. Plexiglas chamber is fixed inside ventilated wooden boxes.

At the beginning of their night cycle, each mouse was subjected to a 30-minute run and data were captured in 5-minute intervals. The photobeam data were analyzed by dividing the open field into a 3x3 grid, forming 9 equal-sized zones. Locomotion was calculated using two open field layouts; one consisted of just the center zone defined as active, and the other consisted of the center and all four corner zones defined as active (Pritchard et al. 2003). Horizontal distance traveled, number of entries and time spent in central and peripheral zones were calculated. Crossover is defined as entries from an inactive to an active zone (Pritchard et al. 2003). Bottom of the chamber is cleaned with Air-Rex in between subjects. PAS Reporter (Software from San Diego Instruments) was used to extract the data into meaningful numerical values.

2.8 Prepulse Inhibition (PPI) of Startle

PPI of Startle is a measure of the animals' sensorimotor gating, by which "*a startle response is suppressed when the intense startling stimulus is preceded by a barely detectable pre-stimulus*" (Braff et al. 2001; Graham 1975). Each animal was placed in a horizontal Plexiglas cylinder that is 5cm in diameter that sits on a white platform inside a ventilated box (SR-LAB, San Diego Instruments, San Diego). Piezoelectric accelerometer is a sensor that sits on the bottom of each platform and was used to measure the animals' movement. Speakers were located 33cm above the cylinder to produce the acoustic stimuli. After a 10-minute acclimation with a white noise background of 65dB, animals were exposed to a random distribution of acoustic stimuli for 15 minutes. These stimuli consist of no tone, 120 dB tone, or a soft acoustic pre-

pulse (69, 73, or 81dB tones) that precedes a 120dB startle tone. Delay between each prepulse and pulse was 100ms. Each chamber was cleaned in between subjects with Air-Rex.

2.9 Fear Conditioning

Fear Conditioning measures the animals' response to a negative stimulus presented in association with an environmental cue (Risbrough et al. 2014). Fear conditioning is a form of associative learning called classical conditioning. Classical conditioning refers to the association of a biologically potent unconditioned stimulus (US) to a previously neutral conditioned stimulus to induce a conditioned response (Bichler et al. 2013; Pavlov 2010). Pavlov's dog is a well-known experiment where he showed how his dogs were able to associate a bell (neutral conditioned stimulus) to food (US). In Pavlov's conditioning study, the dogs began to salivate when they hear the bell ring. In fear conditioning, several dimensions of learning are measured. The first day of the experiment involves allowing the animal to associate a tone (conditioned stimulus) with a foot shock (US), similar to classical conditioning. Context-dependent fear conditioning is measured on day 2, cued-induced fear retention and extinction are measured on day 3, and extinction recall was measured on day 4 (Risbrough et al. 2014).

Four automated Fear Conditioning chambers (MED-Associates, Inc. (St. Albans, VT)) were used with each housing one animal. Each animal was subjected to four consecutive days of measurement with the chamber cleaned between subjects with a

paper towel moistened with water. For Day 1 and 2, animals were habituated for 1 hour under red light with a waterfall tone in a room different from the testing room and were brought into the testing room under a white blanket. In the testing room, a red light and the same tone were used, along with mint scented bedding. For day 3 and 4, animals were habituated for 1 hour in a new room under blue light with a meditation tone and were brought into the testing room using a gray blanket. In the testing room, a blue light and the same tone were used along with almond scented bedding.

Each day is as follows. Day 1 (Acquisition): Animals were acclimated for two minutes followed by three paired tone-shock stimuli with an inter-trial interval of 40 seconds. The acoustic conditioned stimulus (CS: 80dB, 4kHz) was played for 20 seconds in which the last second is paired with a shock stimulus (US: 0.7mA). The shock stimulus was given through a metal grid flooring consisted of 36 rods. Day 2 (Context): Animals were placed back in the same chamber 24 hours later and testing began. Animals were tested for 8 minutes with no tones or shocks. Day 3 (Extinction Training): Animals were placed back into the chambers with a different context (odor, visual, tactile). The grid floor and walls were replaced with a white smooth plastic insert. After 3 minutes of acclimation in which no tones were played, the animals were presented with 50 tones for 30 seconds with an inter-trial interval of 40 seconds. Day 4 (Extinction Testing): Animals were placed back in the same chamber with the same context as Day 3 after 24 hours. After 3 minutes of acclimation in which no tones were played, the animals were presented with 30 tones for 30 seconds with an inter-trial

interval of 40 seconds. Freezing is captured with computer analysis software, Video Freeze (Med Associates, Inc.).

2.10 Statistical Analysis

Statistical analyses were performed by analysis of variance (ANOVA) using SPSS version 23 (SPSS, Inc. (Chicago)) and graphs were made on Prism 6 (GraphPad Software, Inc. (La Jolla, CA)). Strength score from Inverted Grid was calculated using the animals weight (g) multiplied by the amount of the time they held onto the grid (sec). Foot slips and travel time (sec) from balance beam were independently analyzed with ANOVA. %PPI from Prepulse inhibition were calculated using the equation [100-((mean prepulse+pulse)/(mean pulse)) x 100] as previously described (Swerdlow et al. 1992). Percent component time freeze in fear conditioning were extracted from the Med Associate Inc. software and is defined as the time the animal is frozen during the component divided by the total time within that component. Exploratory statistics were used to assess the distribution and skewness of the data. All data that are skewed are transformed using natural log. Data from Inverted Grid, Balance Beam, and Prepulse Inhibition data were analyzed by 3-way and 4-way repeated measures ANOVA (RT-ANOVA) with MIA treatment, time, surgery, and sex as main factors. Significance threshold was set to $p \leq 0.1$, using one-tailed for ANOVA because we hypothesized that MIA would increase inflammation and thereby impair recovery. We therefore hypothesized slower recovery in the MIA TBI groups. Bonferroni's multiple comparisons were used for post-hoc analysis when appropriate (Niesman et al. 2014). Pairwise

comparisons were used to compare groups of each day of measurement to baseline or the first data point. Recovery is defined as the difference between day 49 and day 7, and data were analyzed to determine the magnitude of improvement in the inverted grid and balance beam assay. Grip strength recovery, the decrease in footslip, and the decrease in time to cross the beam were utilized as direct measures of behavioral improvement after surgery. Because we hypothesized a single direction of outcome effect (impaired recovery), one-tailed independent t-tests were used to compare differences between two groups in Graphpad Prism. For all analyses, all data were presented as mean +/- SEM with significance set at $p \leq 0.1$.

Results

3.1 Analyses Approach

Pre-surgical baseline data were analyzed with repeated measures ANOVA as appropriate to the particular behavioral measure, without regard to surgical condition to which the animals were ultimately assigned; this maximized power to detect any effects of MIA before the introduction of surgical intervention status. While it was not possible to balance TBI and Sham group assignments across all of the different behavioral measures for pre-surgery baseline, assignments to groups were balanced according to pre-surgical inverted grid strength scores. In order to minimize the potential for litter effect confounds, no more than two pups of the same sex from a single dam were assigned to a group. In order to balance baseline inverted grid scores across TBI and Sham groups, offspring with high strength scores from the same dam were assigned to Sham and low strength scores were assigned to TBI. Subsequently offspring from the next dam were assigned in the opposite manner. To determine whether any statistically significant assessments of surgery on the different behavioral measures were actually the result of pre-existing group differences rather than the surgical procedure itself, we examined multi-factor ANOVAs on all behavioral measures taken pre-surgery with surgical condition as one of the factors. In the absence of significant main effects of surgery or surgery interaction, post-surgical data was analyzed in its original raw data form, with any significant main effects/interactions with the surgery factor attributable to the surgical intervention itself. Where significant effects of surgery (main effect and/ or interactions) were observed in the pre-surgical data, i.e. prior to the surgical procedure itself, post-surgical data was calculated as percent of pre-surgical baseline for each

animal, and the % baseline values were entered into the appropriate ANOVA design for the post-surgical analysis.

3.2 Inverted Grid

The inverted grid behavioral test was used to determine effects of surgery and MIA treatment on motor grip strength. MIA treatment effect was determined through comparisons between Control and MIA groups. No group differences were observed in baseline grip strength measures assessed prior to surgery (data not shown). Weekly measures were conducted for 7 weeks post-surgery and 3-way ANOVA was done using post-surgery data, as pre-surgery baseline values were unreliable. As shown in Fig. 3, 3-way ANOVA of strength scores identified a within-subject effect of increase with time ($F=68.713$, $p=0.000$), as expected. ANOVA also identified main effects and interactions described in detail below.

3.2.1 MIA Treatment Effect

As illustrated in Fig. 4A, ANOVA of MIA treatment, surgery, and time examining the effect of MIA treatment identified an interaction between MIA treatment and time in grip strength following surgery ($F=2.462$, $p=0.034$). Based on confidence interval of 0.1, pairwise comparisons identified significant differences between control and MIA treatment on day 42 and day 49 ($p<0.1$), as might be expected of an effect emerging over an extended course of recovery. As illustrated in Fig. 4B, further analysis identified a significant time by treatment interaction in sham ($F=2.606$, $p=0.037$) but not in TBI

(F=1.185, p=0.317) surgical groups. 3-way ANOVA did not identify a within-subject interaction of treatment, surgery, and time (F=1.162, p=0.327), and no main effect of MIA treatment was identified by ANOVA (F=0.308, p=0.580). Differences in recovery scores between day 49 and day 7 were next analyzed. As shown in Fig. 4C, independent t-test comparisons did not detect significant attenuation of grip strength recovery in MIA compared to Control offspring (p=0.1718).

3.2.2 Surgery Effect

The recovery of Grip Strength over time in TBI and Sham surgical groups is illustrated in Figure 5. As expected, ANOVA of MIA treatment, time, and surgery identified a main between-subject effect of surgery (F=64.618, p=0.000), Fig. 5A. Repeated measures ANOVA also identified differences in strength scores between Sham and TBI surgical groups through a time by surgery interaction (F=4.108, p=0.001). ANOVA identified significant differences between Sham and TBI animals in both control (F=25.538, p=0.000) and MIA treatment groups (F=41.064, p=0.000). Pairwise comparisons identified significant differences between sham and TBI animals on day 28 ($p<0.1$), and from day 35 to day 49 ($p<0.05$). Of interest, ANOVA identified an interaction between time and surgery in control animals (F=4.600, p=0.001) but not in MIA animals (F=0.850, p=0.511). Strength recovery was calculated using the difference between day 49 and day 7. Magnitude of strength recovery was analyzed for each surgical group, as shown in Fig. 5B, and independent t-test of strength recovery

identified significantly attenuated strength recovery scores in TBI compared to Sham animals ($p<0.000$).

3.2.3 Sex Effect

As an exploratory factor, sex was analyzed in a 4-way ANOVA along with effect of MIA treatment, surgery, and time. 4-way ANOVA identified a significant main effect of sex ($F=11.156$, $p=0.001$). ANOVA also identified a between subject interaction of treatment by surgery by sex ($F=3.048$, $p=0.083$), shown in Fig. 6. Significant outcomes from the inverted grid behavioral measures are summarized in Table 2.

3.3 Balance Beam

Balance and motor coordination was assessed through a balance beam behavioral measure to determine effects of MIA and/or surgery. Each animal was placed on an elevated platform on one side of the apparatus traveling to the other side on a 12mm square beam. The time to travel across the beam and the number of foot slips were measured (Lalonde and Strazielle 2003; Stanley et al. 2005). Weekly measures were conducted up to 7 weeks.

3.3.1 Foot Slip Analysis

Balance Beam performance following TBI or Sham surgery is shown in each of the eight experimental groups in Figure 7. As expected, 3-way ANOVA identified a within-subject effect of time over the course of recovery following surgery ($F=67.526$,

p=0.000). As they recover, they produce fewer foot slips, with a downward trend most clearly seen comparing day 7 to day 49 (Fig. 7).

3.3.2 Foot Slip Treatment Effect

3-way ANOVA of MIA treatment, surgery, and time did not identify any within-subject MIA treatment interactions or a main MIA treatment effect ($F=2.227$, $p=0.138$), Fig. 8A. Of interest, as seen in Fig. 8B, ANOVA identified a significant time by MIA treatment effect in TBI ($F=2.041$, $p= 0.048$) but not in Sham animals ($F=0.332$, $p=0.933$).

3.3.3 Foot Slip Surgery Effect

With groups collapsed across MIA treatment groups, ANOVA identified a main effect of surgery with TBI treatment groups exhibiting higher foot slips than sham treatment groups (Fig. 9A) ($F=335.453$, $p=0.000$). A within-subject interaction of surgery with time ($F=35.677$, $p=0.000$) was also identified. Pairwise comparisons identified significant differences between sham and TBI animals at each measurement between day 7 to day 49 ($F<0.001$), Fig. 9A. Significant main effects of surgery were identified in both control and MIA groups ($F=181.196$, $p=0.000$) and ($F=156.370$, $p=0.000$) respectively. ANOVA found within-subject interactions of time and surgery in control ($F=20.228$, $p=0.000$) and MIA groups ($F=17.454$, $p=0.000$). As shown in (Fig. 9B) the magnitude of recovery, calculated with the difference between day 49 and day 7, is greater in Sham compared to TBI surgical groups ($p<0.0001$). While a greater

magnitude in recovery might suggest the animals have more room for recovery, in this case the TBI animals were more severely injured, suggesting injury severity may have impaired recovery compared to the Sham animals.

3.3.4 Foot Slip Sex Effect

A 4-way ANOVA was analyzed to determine if the factor of sex has any effects and or interactions. ANOVA identified significant main effect of sex ($F=16.509$, $p=0.000$) as well as an interaction with time ($F=2.217$, $p=0.031$) in balance beam performance (between-subjects $F=5.068$, $p=0.026$) (Fig. 10). It can be seen that males have more foot slips than females illustrated in Fig. 10. ANOVA also identified a significant sex x surgery interaction ($F=3.541$, $p=0.062$). Significant findings from balance beam foot slips are summarized in Table 3.

3.4 Balance Beam Travel Time Analysis

As expected due to recovery and training, 3-way ANOVA identified a within-subject effect of time in latency to cross the travel beam ($F=4.306$, $p=0.000$), illustrated in Fig. 11.

3.4.1 Travel Time Treatment Effect

ANOVA did not identify a main effect of MIA treatment in sham ($F=0.000$, $p=0.990$) or TBI animals ($F=2.603$, $p=0.111$).

3.4.2 Travel Time Surgery Effect

Similar to foot slips, there was a main effect of surgery ($F=33.238$, $p=0.000$) and also an interaction with time ($F=7.885$, $p=0.000$) as shown in Fig. 12A, illustrating elevated time to cross the beam in TBI compared to Sham animals. TBI animals started with higher latency to cross the beam at the day 7 testing session, with decreases after day 7 indicating recovery. Pairwise comparisons identified significantly increased time to cross the beam in TBI compared to Sham groups over 7 weeks with the greatest improvement seen in the first two weeks post-surgery ($p<0.001$), differences at week 3 and 7 ($p<0.01$), and differences at week 4 and 5 ($p<0.05$). Further analysis using ANOVA identified significant main effects of surgery in control ($F=10.227$, $p=0.002$) and MIA ($F=24.765$, $p=0.000$). Additionally, there was an interaction of time and surgery in control ($F=5.685$, $p=0.000$) and MIA ($F=3.776$, $p=0.001$). The difference between day 49 and day 7 were analyzed and independent t-test of recovery time, shown in Fig. 12B, identified significantly greater improvement in Sham animals compared to TBI animals ($p=0.0009$).

3.4.3 Travel Time Sex Effect

The additional factor of sex was analyzed in a 4-way ANOVA. The 4-way ANOVA identified a main effect of sex ($F=9.005$, $p=0.003$). Interestingly, it identified a 4-way interaction with time, sex, surgery, and MIA treatment ($F=2.376$, $p=0.025$). As shown in Fig. 13, males have attenuated latency to cross the beam. Significant findings from latency to cross balance beam are summarized in Table 3.

3.5 Open Field

Open field is a test that measures the animals' locomotion in an open chamber, comprised of an open acrylic box. Photobeams were used to track movement and the distance traveled. The amount of time spent in certain defined areas, and the number of entries into those areas were assessed. Data were analyzed for animals from all treatment groups pre- and post-surgery.

Different measurements recorded from this assay were analyzed using repeated measure ANOVA of MIA treatment, surgery, and time interval. A within-subject time effect was identified in total distance traveled ($F=567.674$, $p=0.000$) with locomotion declining as animals acclimated to the environment of the open behavioral chamber. Crossovers, which are entries tabulated when the animal crosses from inactive to active zones, were analyzed. ANOVA for crossovers again identified a within-subject effect of time ($F=416.216$, $p=0.000$) with no MIA treatment effect ($F=0.614$, $p=0.435$). Center duration and center entries were analyzed separately and over the course of the 30-minute experiment, time interval was found significant for both of these measures ($F=20.690$, $p=0.000$) and ($F=42.941$, $p=0.000$) respectively.

3.5.1 MIA Treatment Effect:

Previous studies have not reported significant effects of MIA treatment in distance traveled, and our data was consistent with these prior observations ($F=0.374$, $p=0.541$). ANOVA for crossovers and center entries did not identify any significant effect of MIA treatment ($F=0.614$, $p=0.435$), ($F=0.055$, $p=0.815$) respectively. ANOVA of

center duration identified within-subject interaction with time, MIA treatment, and surgery (F=2.571, p=0.047), Fig. 14A. ANOVA for center duration also identified a time by treatment interaction only in TBI animals (F=2.483, p=0.073) but not in sham (F=1.473, p=0.204). Post-hoc comparisons did not identify any MIA treatment effect between control sham and MIA sham (p=1.000) or control TBI and MIA TBI (p=1.000). Interestingly, the surgery effect in control animals was not significant (p=0.307) but is significant in MIA animals (p=0.004).

3.5.2 Center Entries Baseline Analysis:

Baseline measures were next analyzed to determine if there was a pre-existing surgery effect. ANOVA on baseline values identified an effect of time (F=56.992, p=0.000), and interestingly a 3-way interaction with time, MIA treatment, and surgery (F=3.650, p=0.004). ANOVA also found an interaction of time and surgery (F=2.052, p=0.074). Thus post-surgical data was calculated as a percent over pre-surgical data to determine the effect of TBI and it identified a main effect of surgery (F=17.108, p=0.000) along with an interaction with time (F=3.195, p=0.009), Fig. 15. ANOVA did not find any main effect of treatment (F=0.740, p=0.391) or with any interactions.

3.5.3 Surgery Effect:

ANOVA for total distance identified an interaction between time interval and surgery (F=2.530, p=0.039), Fig. 16A. Interestingly, ANOVA found an effect of time and surgery only in control (F=3.352, p=0.012) and not MIA animals (F=0.476, p=0.757). To

determine if the surgery effect was actually due to the effect of surgery or if it was due to random surgery assortment of the animals, total distance pre-surgery was analyzed. Repeated measures ANOVA did not identify a pre-existent surgery effect ($F=0.035$, $p=0.851$) (data not shown).

Repeated measures ANOVA for crossovers did not find any significant effects of surgery ($F=1.601$, $p=0.208$) or any surgical interactions. ANOVA for center duration identified a main effect of surgery ($F=3.335$, $p=0.070$), Fig. 16B. ANOVA for center entries found a main effect of surgery ($F=14.742$, $p=0.000$) as well as a within-subject interaction with time ($F=3.172$, $p=0.012$), Fig. 16C. While no interactions with surgery were detected in center duration, the effects were identified in center entries. There was a main effect of surgery for both control and MIA animals ($F=3.217$, $p=0.077$) and ($F=15.018$, $p=0.000$); however, ANOVA only identified a time by surgery interaction in the MIA group ($F=1.961$, $p=0.096$) but not in control ($F=1.556$, $p=0.185$), Fig. 16D. TBI has been shown to decrease the number of center entries (Wang et al. 2016).

3.5.4 Sex Effect:

4-Way ANOVA for total distance traveled identified a main effect of sex ($F=4.570$, $p=0.034$), where males travel more distance than females (data not shown). Interestingly, ANOVA of center duration identified between-subjects MIA treatment and sex interaction ($F=5.783$, $p=0.017$) and a within-subjects interaction of time, sex, and treatment ($F=2.501$, $p=0.051$), data not shown. Post-hoc comparisons did not identify any significant MIA treatment effect between any groups ($p>0.3$). 4-way ANOVA for

center entries identified a significant effect of sex (F=3.341, p=0.069). Significant findings from the open field assay are summarized in table 4.

3.6 Prepulse Inhibition of Startle

Prepulse Inibition (PPI) of startle is a measure of sensorimotor gating. The animal will produce a startle response when a loud stimulus is presented. However the startle response will be inhibited when a soft pre-stimulus is presented before the startle stimulus. Prior studies have observed an effect of MIA treatment on PPI (Meyer et al. 2006b; Shi et al. 2003; Wolff and Bilkey 2010). PPI was evaluated for each animal at 3 different time points; before surgery, a week after surgery, and 10 weeks after surgery. %PPI was calculated and analyzed separately at three different pre-pulse intensities through repeated measures ANOVA. A baseline startle magnitude was recorded and analyzed for no before the start of any prepulse stimulus. As expected due to a training effect, repeated measures ANOVA found an effect of time for baseline startle at 120db (F=62.806, p=0.000), %PPI 69dB (F=40.820, p=0.000), %PPPI 73dB (F=69.468, p=0.000), and %PPI 81dB (22.063, p=0.000) (Data not shown).

3.6.1 MIA Treatment Effect:

Due to previous findings of an inhibition of PPI in MIA offspring (Meyer et al. 2006b; Shi et al. 2003; Wolff and Bilkey 2010), it is of interest to analyze pre-surgery data to determine if this inhibition can also be seen. While some studies identified effects of MIA on PPI in males (Fortier et al. 2007; Meehan et al. 2017; Rahman et al.

2017), males and females (Lins et al. 2018; Lins et al. 2019), other studies report MIA treatment effects in females but not males (Vorhees et al. 2012). 3-way ANOVA of MIA treatment, time, and sex at each prepulse identified a significant main effect of treatment ($F=3.410$, $p=0.067$), and a main effect of sex ($F=5.308$, $p=0.023$). Interestingly, ANOVA of %PPI identified significant effects of MIA treatment in female MIA offspring ($F=5.726$, $p=0.019$) but not in males ($F=0.066$, $p=0.797$). Independent t-tests identified a significant MIA treatment effect in Female MIA offspring at the 81dB prepulse ($p=0.0056$), while MIA treatment was close to reaching significance at the 73dB prepulse ($p=0.0562$) (Fig. 17A). In contrast, males did not exhibit an effect of MIA treatment for any of the prepulse intensities ($p>0.9999$).

Post-surgery, repeated measures 3-way ANOVA of MIA treatment, surgery, and time were performed for each prepulse and identified a within-subject effect of time by treatment at 81dB ($F=3.760$, $p=0.027$). It is worth to note that at 63dB, there was a trend towards an effect of treatment ($F=2.254$, $p=0.107$). ANOVA at 81dB identified a time x treatment interaction in sham ($F=3.902$, $p=0.026$) but not in TBI ($F=0.679$, $p=0.502$) (Fig. 17B). Post-hoc comparisons did not identify any significant effects of MIA treatment ($P>0.5$). Repeated measures ANOVA at the 69db, 73dB, and 81dB prepulse did not identify a main effect of MIA treatment ($F=0.129$, $p=0.720$), ($F=0.786$, $p=0.377$), and ($F=0.460$, $p=0.499$) respectively.

3.6.2 Surgery Effect:

It has been previously reported that a deficit in %PPI was observed in TBI animals (Heldt et al. 2014). Repeated measures ANOVA identified a main effect of surgery at both the 73dB ($F=4.699$, $p=0.032$) (Fig. 18A) and 81dB ($F=6.430$, $p=0.012$) (Fig. 18B) prepulse intensities. Repeated measures ANOVA at the 73dB prepulse also identified a within-subject interaction of time with surgery ($F=4.637$, $p=0.011$). Pairwise comparisons identified elevated PPI in TBI surgical groups (Fig. 18), which is the opposite of the %PPI deficit observed in TBI animals previously (Heldt et al. 2014). Further analysis of ANOVA identified main effects of surgery in Control but not in MIA offspring at both 73dB and 81dB prepulses ($F=2.739$, $p=0.102$) and ($F=6.314$, $p=0.014$). It also identified an interaction of time and surgery at the same prepulses ($F=4.224$, $p=0.016$) and ($F=2.385$, $p=0.098$) respectively.

3.6.3 Sex Effect:

ANOVA of baseline startle at 120dB with no prepulse identified a within-subject interaction of time and sex ($F=5.571$, $p=0.004$). A main effect of sex was also identified by ANOVA ($F=45.406$, $p=0.000$) where females exhibit lower startle magnitude than males (Fig. 19A). At the baseline time point, all males had startle magnitudes that range from 125 to 160 whereas the females range from 80-120. Startle magnitudes are computed as arbitrary units that allow further analysis of PPI. A decreasing trend can also be seen in the other PPI sessions (Fig. 19A), in which a decrease in startle amplitude was observed over time. Additionally, repeated measures ANOVA identified a

main effect of sex at each prepulse intensity (F=4.817, p=0.030), (F=17.622, p=0.000), and (F=26.733, p=0.000) respectively. Results are illustrated in Fig.19B, C, and D respectively. ANOVA identified an interaction of time, sex, and surgery at 73dB (F=3.397, p=0.035). It is worthy to note that at the 81dB prepulse, the interaction of time, sex, and surgery was close to significance (F=2.325, p=0.102) (graphic not shown). Last, ANOVA identified a significant effect of sex by surgery (F=3.328, p=0.070). Significant findings from the prepulse inhibition of startle assay are summarized in tables 6 and 7.

3.7 Fear Conditioning

Fear conditioning testing "measures the ability of the mouse to learn and remember an association between an aversive experience and environmental cue" (Crawley 2007). Fear conditioning data was first analyzed for each day of testing by repeated measures 3-way ANOVA with main factors of time, MIA treatment, and surgery. ANOVA identified an effect of time (trial/block) on each day of testing, (F=275.402, p=0.000), (F=13.423, p=0.000), (F=108.712, p=0.000), and (F=83.758, p=0.000) respectively.

3.7.1 Treatment Effect

A 3-way repeated measure ANOVA of MIA treatment, surgery, and block identified a significant treatment effect on extinction training (Day 3, F=4.698, p=0.032), Fig. 20A. Post-hoc comparisons did not identify any significant differences between

groups (p=1.000). Further analysis of ANOVA identified a main effect of treatment in sham (F=4.972, p=0.029) but not TBI animals (F=0.648, p=0.423). Pairwise comparisons identified significant effects of MIA treatment at block 2, 6, and 7 ($p<0.1$) (Fig. 20B). ANOVA did not identify significant time and treatment interactions.

3.7.2 Surgery Effect

Significant effects of surgery were found by 3-way ANOVA at day 1 (acquisition 1, F=9.839, p=0.002) and day 2 (context awareness, F=4.531, p=0.035). ANOVA also identified a time and surgery interaction on day 1 (F=5.046, p=0.009). Further analysis of Day 1 revealed a significant surgery effect in both control and MIA groups, (F=6.341, p=0.014) and (F=3.505, p=0.065), Fig. 21A. Interestingly, ANOVA found an interaction of time and surgery only in control (F=3.067, p=0.055) but not MIA animals (F=2.019, p=0.140). Pairwise comparison identified a significant effect of surgery at trial 2 in control animals ($p<0.01$) and in MIA animals ($p<0.1$). Post hoc comparisons identified surgical differences between control sham and control TBI (p=0.035). Due to the surgery effect found on day 2 by 3-way ANOVA, further analysis using ANOVA revealed a significant main effect of surgery in MIA animals (F=4.938, p=0.029) but not in control (F=0.620, p=0.434), Fig. 21B. Pairwise comparison identified a significant surgery effect in control animals at block 3 ($p<0.1$).

3.7.3 Sex Effect

Significant effects of sex and any interactions of sex were next analyzed using 4-way ANOVA. ANOVA identified main effects of sex on acquisition (day 1, $F=6.196$, $p=0.014$) and on extinction training (day 3, $F=5.016$, $p=0.027$). Along with those findings, ANOVA also identified a trial/block and sex interaction on day 1 ($F=6.085$, $p=0.003$), day 2 ($F=3.273$, $p=0.021$), and day 3 ($F=5.646$, $p=0.019$). Additionally, 4-way ANOVA on day 2 identified a main effect of sex by surgery ($F=3.762$, $p=0.054$), Fig. 22A. Of interest, ANOVA on day 4, found an interaction of time, sex, and treatment ($F=3.712$, $p=0.001$), Fig. 22B. Previous publication gave insight to the significance of sex in fear conditioning (Dalla and Shors 2009) and thus, a 3-way ANOVA of MIA treatment, surgery, and block were analyzed in each sex to determine whether the effects of surgery and treatment were stronger in males or females. It is important to note that since effect of surgery was only observed in males and not females, graphical data were only produced for males are presented in the section below. Significant findings from the fear-conditioning assay using 3-way ANOVA of time, sex, and treatment are summarized in table 8.

3.8 3-Way ANOVA in Males vs. Females

At acquisition (Day 1), the animals were presented a tone associated with a foot shock three times. As shown in the first panel of Fig. 23 and 24, it is evident and as expected that as the animals were trained to associate shock with tone, the amount of freezing increases. As expected, a 3-way ANOVA in females identified a within-subject

effect in trial (F=138.237, p=0.000), but there are no interactions with MIA treatment nor surgery, (F=0.439, p=0.645) and (F=2.130, p=0.122) respectively. ANOVA also did not find any significant between-subject effects; ANOVA did not identify a significant MIA treatment effect (F=1.043, p=0.310). 3-way ANOVA in males similarly identified within-subject effects of trial (F=132.059, p=0.000) and trial by surgery (F=3.988, p=0.022). Post-hoc comparisons identified significant differences between male control TBI and male control Sham (p=0.017). ANOVA did not find significant differences between MIA and control males (F=0.473, p=0.494). When the animals were presented with the initial tone/shock acquisition, the amount of freezing was under 5%. When the second acquisition was produced, %freezing increases. The effect of surgery achieved statistical significance in male subjects (F=11.497, p=0.001) Fig. 25. ANOVA of females did not reach statistical significance (F=1.684, p=0.198) (data not shown). It is to note that on the final acquisition, Sham males experienced the least freezing at 25%, and all the other groups were freezing between 30-40%.

At context awareness (Day 2), animals are placed back into the same environment to determine if they have a behavioral response to any contextual cues. 3-way ANOVA of MIA treatment, surgery, and blocks in females identified a within-subject effect in trial (F=12.752, p=0.000); however, ANOVA did not identify any significant between-subject effects of MIA treatment nor surgery, (F=0.001, p=0.971) and (F=0.013, p=0.908) respectively. In contrast, a main effect of surgery (F=7.971, p=0.006) and a within-subject effect of trial (F=3.154, p=0.026) were identified in males, as seen in the second panel of Fig. 25. What is worthy to note, is that the effect of TBI is

most clearly seen in MIA males, who showed an increase in freezing compared to the Sham males throughout all four days of the experiment.

At extinction training (Day 3), the animals were placed into a new environment with tones playing. The goal was to assess the conditioned response when a tone is played. Baseline values were not included in the analysis using ANOVA. Similar to the first two days in females, a 3-way ANOVA identified a within-subject effect of trial ($F=24.564$, $p=0.000$) but effects of MIA treatment and/or surgery were not found. Main effects of MIA treatment almost reached significance ($F=2.982$, $p=0.088$), which can be seen on the third panel of Fig. 23. In both Sham and TBI groups, there was a trend towards lower freezing in the MIA compared to Control offspring, which did not reach statistical significance. Pairwise comparison found significant differences between MIA sham females and control sham females ($p<0.1$) at block 7. In males, a 3-way ANOVA identified an expected within-subject trial effect ($F=26.266$, $p=0.000$) and more interestingly unlike the first two days, the main effect of surgery did not reach significance; however, it was close to significant ($F=3.099$, $p=0.082$). MIA treatment again did not reach statistical significance ($F=1.788$, $p=0.185$), third panel of Fig. 24 and 25; however, it is worthy to note that there is a trend for MIA/Sham males freezing less compared to control/Sham males ($F=3.075$, $p=0.087$).

At extinction recall (Day 4), 3-way ANOVA in females identified a within-subject effect of trial ($F=18.029$, $p=0.000$), but no main effects of MIA treatment nor surgery were found, ($F=2.164$, $p=0.145$) and ($F=0.113$, $p=0.738$) respectively. Even though a between-subjects MIA treatment effect was not identified by ANOVA, pairwise

comparisons identified MIA females exhibiting less freezing than control in block 6 ($p<0.05$), (Fig. 23 panel 4) where there is a separation between MIA treatment and control. In males, a 3-way ANOVA also did not find an effect of MIA treatment nor surgery, ($F=0.532$, $p=0.468$) and ($F=1.555$, $p=0.216$) respectively. Similar to the females, the males showed a within-subject trial effect ($F=24.800$, $p=0.000$). This trial effect can be seen on the fourth panel of Fig. 24 and 25. The trend starts off high after baseline freezing was measured and then tends to even out throughout the rest of the session.

A comparison between surgeries in MIA males revealed an interesting finding. As Fig. 25 shows, MIA/TBI animals freeze more than MIA/Sham animals throughout all four days of the experiment; hence total freezing for all four days were summed for each surgical groups in MIA males, Fig. 26. It is to note that the surgery effect in males is clearly seen in MIA animals; however, ANOVA did not identify any significant MIA treatment differences. Independent t-test in MIA males identified significantly greater freezing in TBI compared to Sham mice ($p=0.0059$). Significant findings from the fear conditioning assay using 3-way ANOVA are summarized in table 9.

Discussion

The synergistic effects of MIA and TBI found in this study have implications for millions of patients affected by TBI annually. Many have found *in utero* exposure to inflammatory stimulation results in long-standing alterations in systemic inflammatory response in MIA offspring (Arrode-Bruses and Bruses 2012; Garay et al. 2013; Meyer et al. 2005; Missault et al. 2014; Onore et al. 2014; Ozawa et al. 2006; Patrich et al. 2016; Patterson 2002). The acute outcome of TBI is mediated through inflammation (Doble 1999; Dong et al. 2009; Hynd et al. 2004; Ong et al. 2013). Additionally, evidence supports the hypothesis that long-standing neuroinflammation from TBI worsens with time and can lead to psychiatric disorders (Chodobski et al. 2011; Hiebert et al. 2015; Lozano et al. 2015; Witcher et al. 2015; Woodcock and Morganti-Kossmann 2013). Because outcome following TBI is significantly influenced by inflammation, we were interested in determining if MIA has effects upon the outcome of TBI. Our data identified statistically significant effects of MIA, in several behavioral outcome measures of recovery following TBI. Our results identified deficits in recovery after Sham or TBI surgery in MIA animals compared to Control mice. ANOVA of inverted grid data identified a significant Time x Treatment interaction [($F=2.462$, $p=0.034$), Fig. 4A]. Interestingly, ANOVA of inverted grid behavioral data identified the MIA treatment effect in the milder sham surgical animals ($F=2.606$, $p=0.037$) but not in the more severe TBI surgical groups ($F=1.185$, $p=0.317$). In contrast, ANOVA of the balance beam foot slips behavioral measure identified significant effects of Time x Treatment in TBI ($F=2.041$, 0.048) but not in sham surgical groups ($F=0.332$, $p=0.933$).

4.1 Synergistic Effects of MIA and TBI on Motor Function

To the best of our knowledge, there are no prior studies on the effect of MIA and TBI on motor functions. Here we discovered an interesting interaction between TBI and MIA. In the inverted grid assay, the effect of MIA affected grip strength in sham animals ($F=2.606$, $p=0.037$), but not in TBI animals ($F=1.185$, $p=0.317$), as shown in Fig. 4A. Interestingly, our results observed a time by surgery effect in control animals ($F=4.600$, $p=0.001$) but not MIA animals. This may imply a synergistic effect between TBI and MIA. Similarly, in distance traveled of the open field assay, there is a surgery effect seen in control animals but not in MIA. Our prepulse inhibition of startle results, at the 81dB prepulse, showed a MIA treatment effect in sham animals ($F=3.902$, $p=0.026$) but not in TBI ($F=0.679$, $p=0.502$), Fig. 17B. This is further supported through a surgery effect at the 73dB and 81dB prepulses that is again seen in control animals but not in MIA animals. Additionally, day 1 of fear conditioning show that TBI animals, in both control and MIA, freezes more than sham (Fig. 21A). Overall, our data suggests a ceiling effect. The impact from TBI alone, has already reached its pinnacle and the effects of MIA is not able to make it measurably worse.

In contrast, center entries of open field identified a surgery effect in MIA animals but not in control animals, Fig. 16D. This finding supports our hypothesis of a more severe TBI effect in MIA animals. However, we see contradicting results in our fear conditioning day 2 result. A study by Wang et al., discovered that prenatal infection exposure causes hippocampal injury and memory deficits, which is paradoxical to our data because we see an increase in %freezing. Their finding was also supported by the

volume of the hippocampus that was significantly reduced in MIA animals compared to control (Wang et al. 2013). Therefore, the damage caused by TBI may have a more detrimental effect on MIA animals as they already sustained life-long hippocampal injuries. Contrarily, MIA offspring has been shown to have corticolimbic brain impairment, in which MIA animals have an increase in freezing behavior in response to context (Sangha et al. 2014). In our data, MIA/TBI has increased %freezing than MIA/Sham. This implies that the TBI surgery has an effect on MIA animals; however, due to %freezing in control animals to be quite similar, it could also imply that the sham surgery affected the MIA animals in such a way that it may have led to decreased freezing. To determine the effect of sham surgery on MIA animals, additional experiments would be needed. This can be done with groups of control and MIA animals that did not get the sham or TBI surgery. This would allow us to determine the severity of the sham surgery on these animals.

4.2 Mechanism of MIA/TBI Effect on Motor Function

Evidence suggests IL-6 is a major mediator for motor coordination deficits following TBI, as it modulates the degree of neuroinflammation (Hergenroeder et al. 2010; Minambres et al. 2003; Yang et al. 2013a; Yang et al. 2013b). In Yang's study, blockade of IL-6 improved the motor coordination deficit caused by TBI, implying that IL-6 may affect the animal's performance in assays in which their coordination plays a role. Thus since IL-6 was found to be sufficient to cause phenotypes similar to MIA (Patterson et al. 2008; Smith et al. 2007) and TBI-induced IL-6 was found to mediate

motor coordination (Yang et al. 2013a), we speculate MIA animals with TBI to produce more foot slips. Interestingly, our MIA animals with TBI have slightly more foot slips than control with both forms of surgery as illustrated in Fig. 8. From literature, IL-6 mediated motor coordination and this deficit was corrected when IL-6 was blocked (Hergenroeder et al. 2010; Minambres et al. 2003; Yang et al. 2013a; Yang et al. 2013b). This may imply that the MIA offspring may have more endogenous IL-6 that contributes to one of the reasons how it exacerbates the inflammation caused by TBI; however, further experiments will be needed to determine if MIA/TBI animals have more IL-6 than control TBI animals. One recent study has found increased basal IL-6 levels in both serum and in the brain of MIA offspring, with an even greater enhancement in IL-6 levels following inflammatory stimulation (Hsueh et al. 2018). These findings suggest increased IL-6 in response to TBI as a plausible mechanism contributing to exacerbation of TBI outcome in MIA offspring.

4.3 Effects of MIA

While effects of MIA were observed in several of our behavioral assays (as detailed above), statistically significant effects of MIA were not detected in all of our assays. Previous publications did not find significant differences between Poly I:C induced MIA and control offspring in fear conditioning (Zuckerman and Weiner 2005); however, our data suggest that MIA has an effect on day 3: Extinction training. As illustrated in Fig. 20, MIA seems to have an effect in sham animals, an interesting finding that is consistent with our inverted grid results. Our data for the other days in

fear conditioning, did not find any significant effects of MIA, which raises a question of whether MIA affects their mental process of extinction. In the open field assay, many publications have investigated the effects of MIA in locomotion and have observed variable results. While our laboratory (Bronson et al. 2011; Richtand et al. 2011; Richtand et al. 2012a; Vorhees et al. 2012) and others (Hsiao et al. 2012; Koenig et al. 2005; Van den Hove et al. 2006) have not observed effects of MIA treatment in total distance traveled, we have also reported dose-dependent effects of poly I:C on open-field locomotor response in MIA offspring (Vorhees et al. 2015), while some have found a significant reduction in distance traveled (Moreno et al. 2011; Smith et al. 2007; Xuan and Hampson 2014). Our results did not show any MIA treatment effects in center entries nor total distance traveled; however, our center time analysis identified a time x surgery x treatment interaction ($F=2.571$, $p=0.047$). Our results show that all four groups spend different amount of time in the center (Fig. 15). Because it has been shown that MIA offspring spend less time in the center zone (Shin Yim et al. 2017) and along with our data, it may suggest a synergistic effect of TBI and MIA.

4.4 Involvement of Sex in Surgery and Treatment

Although the factor of sex was not part of our original hypothesis, it was observed to have an impact on several results in our assays. Notably, the surgery effect found only in sham animals and not MIA animals involves the sex as we see a 3-way interaction of sex, treatment and surgery ($F=3.048$, $p=0.083$). In time to cross the balance beam, we see a significant 4-way interaction of time, sex, surgery, and

treatment (F=2.376, p=0.025). What is interesting to note is that without the factor of sex, the interaction of time, surgery, and treatment did not reach statistical significance. This may suggests that if there is a synergistic effect between MIA and TBI, it might differ between the sexes.

4.5 Effects of Sex and MIA Treatment Interaction

Previous literatures have speculated that reductions in both center duration and center entries may correlate to greater anxiety (Bailey and Crawley 2009; Crawley 1985). Here we observed an interaction with time, sex, and treatment (F=2.501, p=0.051). This was supported by a main effect of sex and treatment (F=5.783, p=0.017). Many others have found similar results in which MIA offspring do not spend as much time in the center as they tend to stay more in the outer perimeter (Hsiao et al. 2012; Meyer et al. 2006a; Smith et al. 2007). However, we observed a reduction in center duration, and no change to center entries. It is possible that MIA causes alterations in exploratory behaviors differently in males and females. Previous publications have found that females have more locomotion than males (Johnston and File 1991) meaning more exploratory activities. In our data, we have observed the opposite; females traveled less (data not shown). This may be from experimental differences between our approaches as we used state of the art equipment compared to those used in 1991. Our data suggests that MIA works differently in males and females. To the best of our knowledge, no other study has looked at the effect of sex after TBI, and thus sex differences after surgery may need to be further investigated.

Previous studies have shown greater freezing in TBI groups compared to Sham (Reger et al. 2012). Our data identified an interaction of time, sex, and treatment ($F=3.712$, $p=0.001$) and we observed that MIA females display lower freezing than control females and this was not seen in males, Fig. 20B. As mentioned earlier and in Table 1, the hippocampus is one of the regions that are affected upon CCI surgery. A possible explanation could be that hippocampal damage impairs the contextual aspect of an experience, and thus learning of an association is impaired (Banta Lavenex and Lavenex 2009; Lavenex et al. 2006; Mumby et al. 2002).

Some (Boksa 2010; Klein et al. 2013; Mattei et al. 2014; Patterson 2009; Smith et al. 2007; Wolff and Bilkey 2010) but not all (Lins et al. 2018; Missault et al. 2014; Van den Eynde et al. 2014; Vorhees et al. 2015) have observed a deficit in PPI in MIA offspring. Our pre-surgery data identified a paradoxical finding in which a deficit in %PPI was found at the 81dB prepulse only in female MIA animals (Fig. 17A). Other studies have observed the opposite in wildtype rodents (Kinkead et al. 2008; Plappert et al. 2005), MIA rodents (Lins et al. 2018; Lins et al. 2019), normal human subjects (Swerdlow et al. 1997), and schizophrenia patients (Kumari et al. 2004).

Prepulse inhibition deficits in MIA females have been previously documented in MIA literature (Vorhees et al. 2012; 2015). We observed similar data that showed a PPI deficit in females across treatment groups at each prepulse as well as a diminished uninhibited startle response. This effect was pronounced and was observed at all three time points. A possible explanation for this effect could be the estrous cycle in female mice. One study has found reduced PPI in female rats during the proestrous phase

(Koch 1998) and another study found males mice to show increase PPI compared to females premenopausal (Ison and Allen 2007). Relating to humans, Swerdlow et. al found significantly reduced PPI in women during different phases of their menstrual cycle (Jovanovic et al. 2004; Swerdlow et al. 1997). These studies suggest that the estrous cycle may have an effect on the reduced PPI that we observed; however, in our study the estrous cycle was not factored into our analysis.

One interesting finding we observed was on day 4: Extinction testing, in which the animals were tested to see if they will freeze upon hearing a tone that initially was paired with a foot shock. One study observed increased conditioned fear in MIA females C57BL6/J mice and not so much in males (Schwendener et al. 2009). In their study, they have 3 days of fear conditioning and extinction was tested once. In each day of the experiment, they found that female MIA mice freeze more than control. Their male group showed slightly similar effects but is more robust in females, in which effects of MIA can clearly be seen between offspring of control and MIA. In our study, we observed an effect of MIA treatment in females but not males in Extinction testing, Fig. 22B. A possible explanation could be that MIA females are more sensitive to adverse conditions in comparison to males. If this was the case, then our data makes sense because MIA females were observed to have increased in fear-like behaviors (Weinstock 2007).

4.6 Effects of TBI

As expected, our results showed significant effects of both Sham and TBI surgery upon alteration to the animals' behavior. We observed profound grip strength deficit in TBI subjects a week following surgery with minimal recovery over the next 7 weeks (Fig. 5). Hence the surgery effect observed supports previous data that CCI is an effective method for inducing a consistent TBI (Levchakov et al. 2006; Mao et al. 2010a; Mao et al. 2010b) as the injury does not improve over time causing long-term functional deficits (McAllister 2008). Also consistent with previous findings (Russell et al. 2011), our TBI subjects showed an increase in foot slips (Fig. 9). As previously mentioned, the outcome of TBI is mediated through inflammation (Doble 1999; Dong et al. 2009; Hynd et al. 2004; Ong et al. 2013). CCI surgery was able to produce profound results that can be observed across multiple levels of analysis despite eliminating variables such as sex and or MIA treatment. The higher number of continuous foot slips observed in TBI animals throughout the experiment imply that TBI animals do not fully recover and sustained long-term impairment.

Additionally, results showed that TBI animals travel more than Sham animals (Fig. 14B). We found no effects of surgery in the crossovers. Since one of the areas that were affected by CCI surgery is the motor cortex, we assumed TBI animals would travel less. It has been found by a few publications that no effects of TBI on exploratory behaviors were found (Malkesman et al. 2013; Wagner et al. 2007; Washington et al. 2012); however, some have found the opposite in which, TBI subjects have an increase in mobility (Budinich et al. 2013; Schwarzbold et al. 2010). This increase in mobility,

which is also seen in our results (Fig. 14B), has been speculated to be an impairment to recollect spatial memory (Schwarzbold et al. 2010), leading to a decrease in the normal attenuation of locomotor response as the environment becomes more familiar.

Reductions in both center duration/entries have been speculated to have a correlation to greater anxiety (Bailey and Crawley 2009; Crawley 1985). In the present study, we observed a reduction in center duration and center entries in TBI subjects (Fig. 14A and 14B respectively). Our findings are consistent with previous studies, which also found a reduction of center duration (Jones et al. 2008; Malkesman et al. 2013) and center entries in TBI subjects (Wang et al. 2016).

A PPI deficit has been observed in patients with schizophrenia (Braff et al. 2001); and has also been observed in MIA offspring (Meyer et al. 2006b; Shi et al. 2003; Wolff and Bilkey 2010), however, we did not see it in both sexes in our result. Our PPI data has demonstrated an effect of TBI at higher prepulses, 73dB and 81dB (Fig. 18), which is not consistent with previous TBI studies (Washington et al. 2012). In their study, they found a trend towards lower %PPI in animals with TBI; however, it did not reach statistical significance. As previously mentioned, we observed higher %PPI in TBI subjects than shams at multiple prepulse intensities, with statistically significant surgical group differences reaching significance at week 10 (Fig. 18). This could be from experimental differences between the two studies. In the study with Washington et al., %PPI was measured 20 days after surgery; which is the closest to our week 1 measurement. Due to variability in the results obtained in day 20 of their study, it could be that our study was actually quite similar and if they would have continued

measurements closer to week 10, they may have also seen significant group differences. Another possibility would be the effect of MIA acting on the damage caused by TBI. To the best of our knowledge, this is the first study to introduce MIA to the outcome of TBI. Therefore, since we observed the opposite of a decreasing trend in % PPI in TBI animals, which has been previously published (Heldt et al. 2014), MIA could be causing this increase in %PPI in TBI animals. Although this can be a very plausible explanation, it warrants more experiments to be investigated.

4.7 Effects of Sex and TBI surgery

The interaction between sex and surgery was found in multiple assays in our experiment. In foot slips of balance beam, there was a main effect of sex x surgery ($F=3.541$, $p=0.062$). As illustrated in Fig. 10, TBI males produce more foot slips than TBI females. The same trend can be seen in sham animals but is not as pronounced. This could suggest that male mice's coordination is more affected than females. More coordination experiments would be needed to fully conclude how much more severe male mice are compared to females.

4.8 Report of Sex Differences

Although sex differences were not part of our original hypothesis, we have found some compelling results suggesting differences between the sexes. Our results indicated a sex effect in many of our assays but in the balance beam assay, our findings were not consistent with previous publications (Mychasiuk et al. 2014; Russell et al.

2011). We observed that males produce more foot slips and take longer to cross the beam. One contributing factor could be the animals' size. Males are generally bigger than females and since the beam was not corrected to the animal's size, it may be in favor of females to perform better than males. Publications have also found that female MIA mice produced more foot slips than male when they are 3 months old (Bakos et al. 2004). Our results could not replicate the same results in the females; however, we demonstrated that MIA and control mice performed equally. We also demonstrated differences between sexes in grip strength, with females generally hanging onto the grid longer than males across all surgery and MIA treatment groups. Again, this may be explained by the difference in size between the two sexes. Sex difference in recovery from surgery may need further investigation.

Our data presented a significant effect of sex in acquisition, context awareness, and extinction, suggesting differences in association learning between the sexes. Comparison between males and females show that females freeze more than males. Multiple studies have looked at the effect of sex in fear conditioning and have found conflicting results. Our results on acquisition day showed that males acquired associations slower than females. However, males acquiring associations faster than females has also been previously observed (Dalla and Shors 2009). Estrogen in females and testosterone in males have been suggested as contributing factors to sex differences seen in fear conditioning (Dalla and Shors 2009).

Overall, we found no effects of sex on surgery outcomes; however, on a recent 2019 study, Doran et. al found significant sex differences that impact males more than

females (Doran et al. 2019). Additionally, animals with Hypoxia Ischemia (HI), a condition in which the brain injured from decreased oxygenated blood, were tested and they did not find significant sex differences (Hill et al. 2011).

4.9 Study Limitations

In the Inverted Grid assay, strength score is a product of weight and grip time, and thus lighter animals must grip longer to have a similar score. This measure may not scale perfectly and lighter animals may have an overall advantage. Our baseline grip strength values could not be used due to an error in application of the assay technique resulting in unreliability in baseline values. Therefore our results were analyzed with the initial post surgical time point (day 7) used as the baseline data point. An alternative strength test such as forelimb grip strength (Bonetto et al. 2015) could be used instead to allow detection of a wider range of individual differences. This grip strength test works by using a commercial automatic grip strength meter, in which the animal is gripping onto the meter while being pulled away from it (Bonetto et al. 2015). It measures the maximum resistance force when the animal is being pulled. By using this assay, it may provide a more detailed index of individual differences.

The injury induced by the CCI in our experiments was limited to only one brain region. It is possible that the site of injury did not affect certain motor behaviors. Cerebellum is an important area of the brain that controls movement and balance (Morton and Bastian 2004). Many areas were injured from the CCI but the cerebellum was not since its location is at the back of the brain. A cerebellar injury could potentially

have a larger affect upon the results of our balance beam assay. The severity of the injury was also a fixed injury, which means that each animal received the same degree of injury. Since our interest was to identify differences in recovery following TBI, it is possible that the severe injury given to each animal is too severe to allow a robust range of recovery to their behaviors. Perhaps conducting behavioral assays with different severities of TBI and determine its effects would give more insight to how it would affect behavior. Interestingly, since MIA has altered inflammatory response, a more severe TBI could influence a more profound synergistic effect that can be seen in their behavior.

In addition, in-vitro assays were not performed after the behavioral assays due to timing limitation. However, since our study is a novel study that was never studied before, it is of interest to assess the behavioral aspect first before testing on a molecular scale. It is possible that MIA treatment differences can be seen in a molecular scale that was not seen in behavioral experiments. MIA offspring has been shown to have differences in cytokines in particular brain regions (Garay et al. 2013) thus it is of interest to harvest different brain region and analyze cytokine expression in each treatment groups.

It is important to note that the overall %freezing in our data is lower than what publications have previously found. It is speculated that a contributing factor is due to the animals' age; however, suppression of contextual memory was seen in mice of 8-12 months and no differences can be seen at 2-6 months (Shoji et al. 2016). Our mice were approximately 4.5 month old when the fear-conditioning assay was performed,

which is in the range where no differences in %freezing between groups can be seen. Therefore, it is not known why our animals show lower %freezing. Another contributing factor could be because the animal already has a high level of stress due to each animal going through multiple different assays of increasing stress. Since fear conditioning was performed last, the high level of stress could have lowered the freezing response.

4.10 Clinical Implications

Treatment intervention for TBI is complex and may not work for everyone. Understanding the different mechanisms for each individual will not only provide proper treatment but also how recovery is altered in those who are MIA patient. Here we present for the first time that a possible comorbidity of MIA and TBI and how it influenced the rate of recovery. Recognizing this synergistic effect may allow the possibility to find or develop drug treatments to increase the rate of recovery for individuals. However, these mechanisms involved may be different in individuals with a history of prenatal stress compared to those without. Thus, it will be beneficial to determine if there is more than one mechanistic pathway that modulates recovery in MIA patients with different backgrounds. The data presented may also serve as an initial step to discover underlying mechanisms for research using MIA as a model to study TBI.

4.11 Public Health Implications

Environmental factors can activate the maternal immune system, which can alter offspring brain development. TBI is a common public health problem, afflicting 10 million people annually (Hyder et al. 2007). Our data represent an initial step in establishing a connection between MIA and TBI inflammatory responses, which with further study, can help determine what behavioral outcome is more affected than others. Doing so will allow patients to receive proper care and treatments. Conceivably, a screen for MIA in patients with head injury could help identify those that are of risk and would require different treatment. The prepulse inhibition of startle assay might be considered as an additional tool, since MIA offspring has been shown to exhibit a deficit in this measure (Meyer et al. 2006b; Shi et al. 2003; Wolff and Bilkey 2010). Thereafter, it will be of interest to establish risk factors and awareness for mothers to prevent immune activation. This can achieve more informed medical decisions during pregnancy, with greater emphasis placed on risks of infection. Precaution for falling and head injuries should also be established or taught at school because it can ultimately affect one's quality of life and their family.

Finally, further study will be needed to extend public health application through determination of similar outcomes during different pregnancy stages; perhaps with using LPS to mimic a bacterial infection; using mammals instead of mice; and in response to different cytokines.

4.12 Future Directions

In the present study, only motor and non-motor behaviors were assessed with no additional assays after. If measurements of behavioral outcomes were repeated, many of the assays can be modified or replaced with others that may produce additional results of interest. In balance beam, we have seen that when animals become over trained, they may become uncooperative and unmotivated to cross the beam (Luong et al. 2011). A modified balance beam, in which the animals will be walking across an incline beam instead of a horizontal beam can be used to motivate animals to cross (Carter et al. 2001).

In place of the open field test, an elevated + maze can be utilized as another way to assess anxiety. This test can be used because confining the animal in open arms of the apparatus is correlated to significantly more anxiety related behaviors (Pellow et al. 1985). Furthermore, a repeated measure for the elevated + maze may allow us to track anxiety levels before and after surgery, as well as how anxiety levels may change over time as the animal recovers. This is doable because it was found that repeated testing has no significant impact on measures of anxiety related behaviors (Hagenbuch et al. 2006; Pellow et al. 1985).

In addition to using prepulse inhibition of startle, an electroencephalogram can be use to measure the activity of sensorimotor gating. It was found that activity of the sensorimotor gating could be measured in the CA3 region of the hippocampus (Curzon et al. 2009; Freedman et al. 1983). This additional test may serve as an additional test

to determine group difference because %PPI is a ratio calculation that is sensitive to changes in the denominator.

Another approach to determine an MIA treatment effect could be sacrificing animals at different time points and measuring the amount of immune cells expressed in the brain. This can potentially give insight into the degree of inflammation different MIA treatment groups may have. Using histology, we may study brain structures before (MIA and control) and after TBI (TBI MIA and TBI control). This can give insight into developmental alterations caused by MIA and/or TBI. RNA sequencing is another technique that will allow the entire transcriptome to be analyzed for differences between MIA treatments. This can be done before and after the dams are injected with either Poly I:C or saline. This will allow us to see differences in gene expression that are either elevated or reduced after injection. RNAseq can also be done before and after surgery to determine differences. Once differences are determined, it is possible to find models with the gene knocked out and tests if the genes that are normally upregulated in Poly I:C treated dams can prevent the effects on the offspring. IL-6 is a good example that can be knocked out because of its relevance in motor coordination as well as its' sufficiency to cause similar phenotypes as observed in MIA with prenatal administration (Patterson et al. 2008; Smith et al. 2007). Assays such as ELISA, Western Blot, or RTqPCR can be utilized to determine which inflammatory markers are elevated or reduced.

Additionally, since our data suggests attenuated recovery in MIA animals after TBI, it would be interesting to further study the effects on recovery using drug treatment.

Anti-inflammatory drugs could be administered through the animals' food, water, or through injections. With this experiment, it is possible to determine if different anti-inflammatory drugs can increase the rate of recovery. Furthermore, an FDA approved library can be used to screen for potential drugs that can help recovery. From there, we can use different techniques to further study how this compound affects recovery. For example, if the binding site of this compound is specific or non-specific. It is also possible to determine its effects on the RNA, DNA, and protein level.

The animals used in this study were limited and gave a small sample size; however, our preliminary results in inverted grid and PPI, show that MIA affects the magnitude of recovery. In balance beam and fear conditioning, a trend can be seen towards a similar direction. Thus, it would be beneficial do a power analysis to determine how many animals are needed to see the extent of the effect. Since CCI was sufficient to injure the animal and surgery effect can be seen across behavioral assays, there is no need to increase the severity of the injury. One of the concerns is that a more severe impact to the brain can potentially kill the animal. However, it is interesting to impact a different area of the brain such as the whole hippocampus to study the effects of memory (Broadbent et al. 2010). It would be interesting to study how injury to this region regulates memory across different experimental conditions. With the current site of impact affecting several areas of the hippocampus, it would be interesting to perform the Morris water maze on the mice to test the effects of MIA on learning and memory recovery following from TBI (Harrison et al. 2009).

Figures

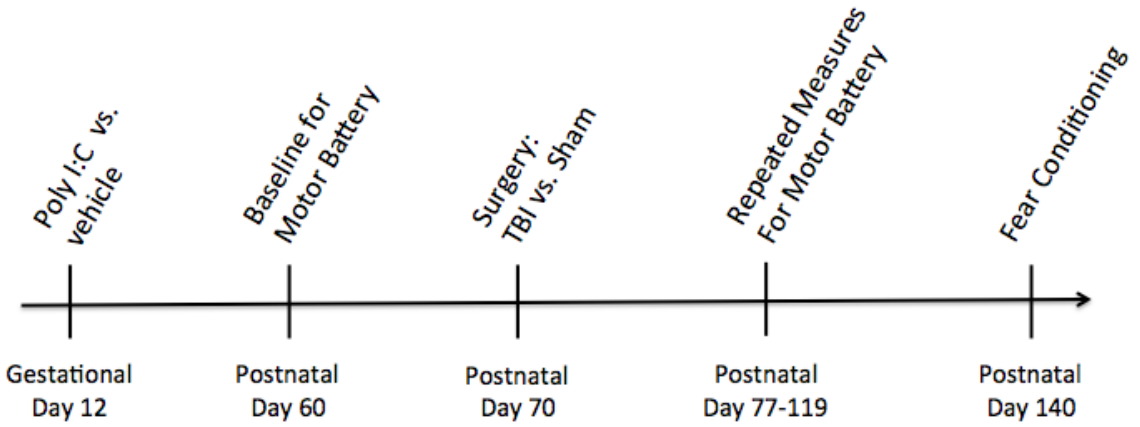
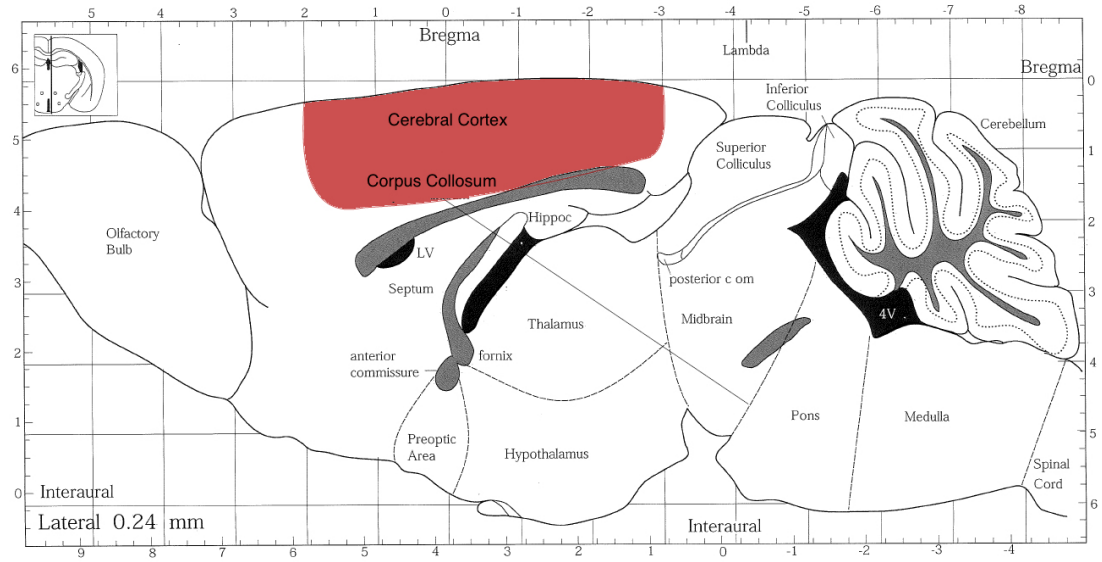


Figure 1. Experimental Design Summary
Pregnant dams were given MIA treatment on Gestation Day 12. Baseline measurements began after offspring were mature on Postnatal Day (PD) 60. TBI vs. Sham surgery was performed upon completion of baseline measures around PD70. Repeated behavioral measures were conducted for 7 weeks and Fear Conditioning was performed on 10 weeks post-surgery.

A



B

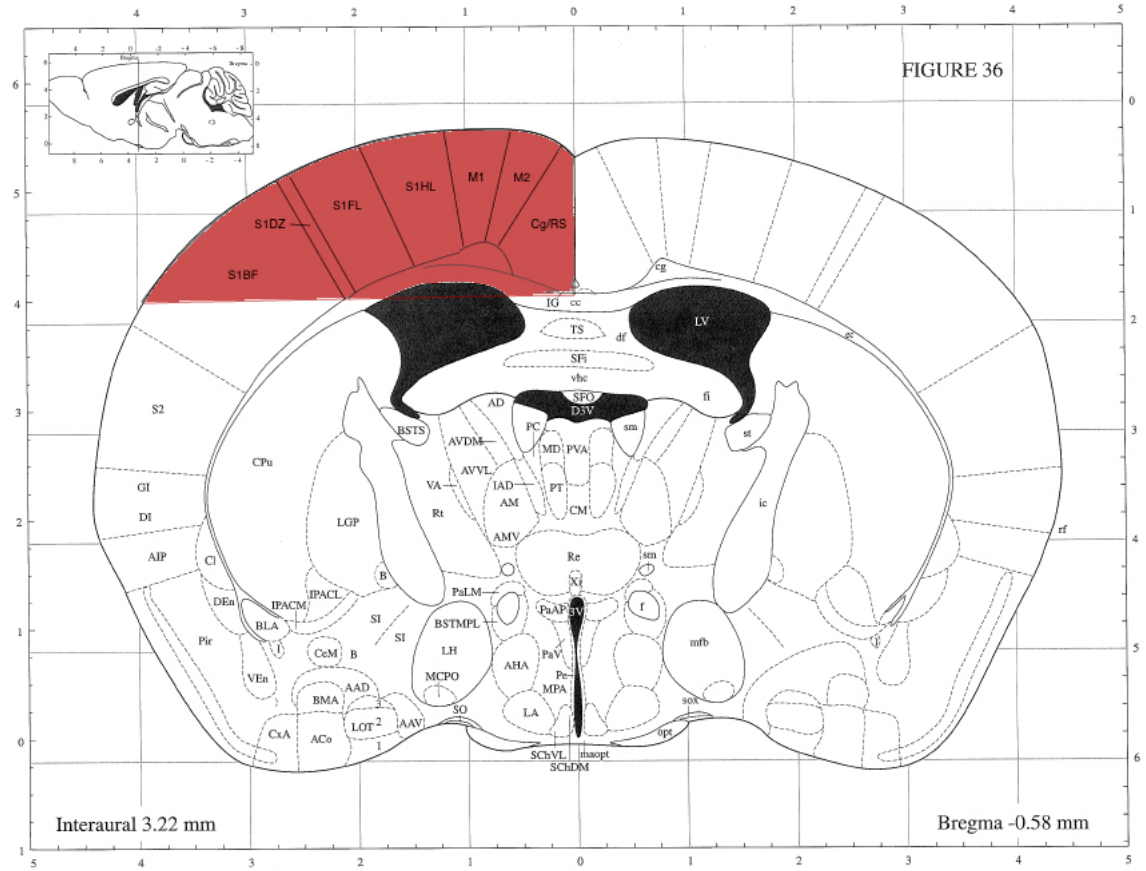


Figure 2. Site of Injury from Controlled Cortical Impact

A craniotomy was created using bregma as a starting point with approximate dimensions 3mm upper width, 4mm lower width, and 5mm length. Site of impact may include but not limited to motor cortex, auditory cortex, visual cortex, somatosensory cortex, retrosplenial cortex, and the hippocampus. Images taken from (Paxinos and Franklin 2004).

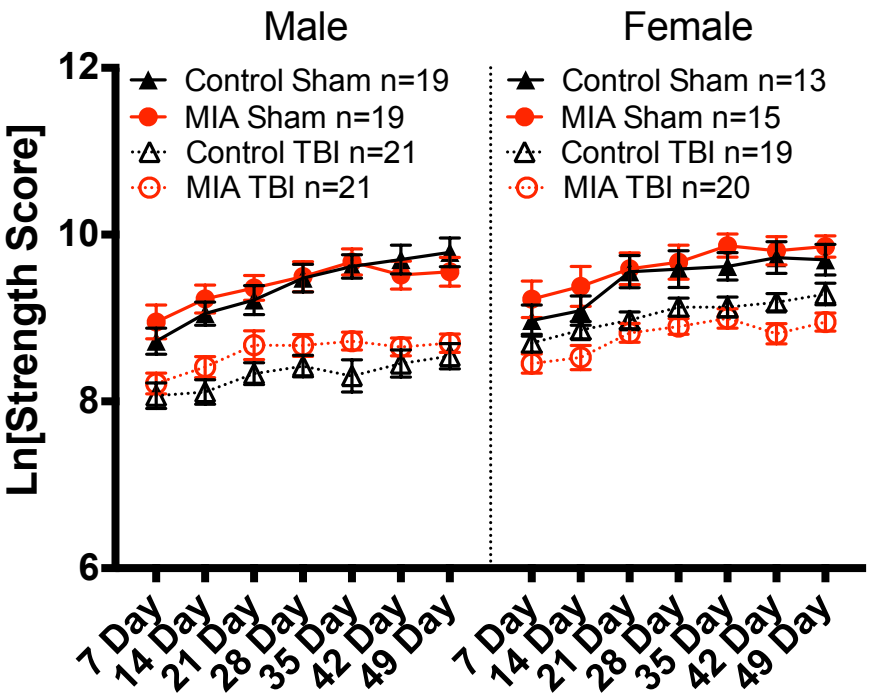


Figure 3. Overall Performance of All Experimental Groups on Inverted Grid. All MIA treated and TBI groups are graphed separated by sex to show overall performance in Inverted Grid. 3-way ANOVA identifgied a significant effect of time, with increase in strength score indicates recovery over time (F=68.713, p=0.000).

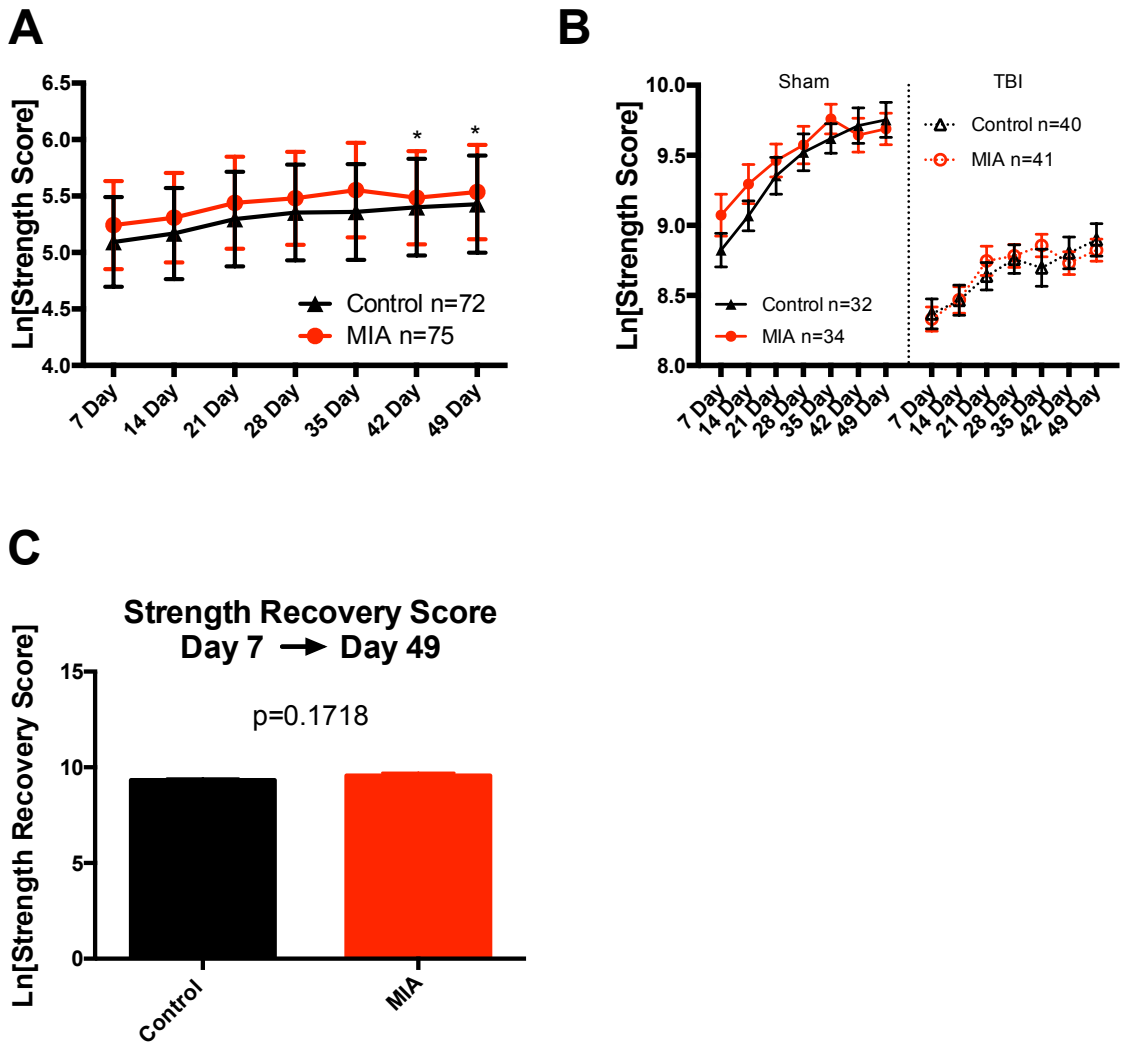


Figure 4. MIA Significantly Attenuated the Magnitude of Grip Strength Recovery from Surgery. (A) ANOVA identified an overall significant interaction between time and MIA treatment ($F=2.462$, $p=0.034$). (B) Further analyses by ANOVA identified a significant time by treatment interaction in sham ($F=2.606$, $p=0.037$) but not in TBI ($F=1.185$, $p=0.317$) surgical groups. (C) Independent t-tests did not identify differences between control and MIA treatment in overall grip strength recovery from (TBI and Sham) groups over 7 weeks of testing ($p>0.1$).

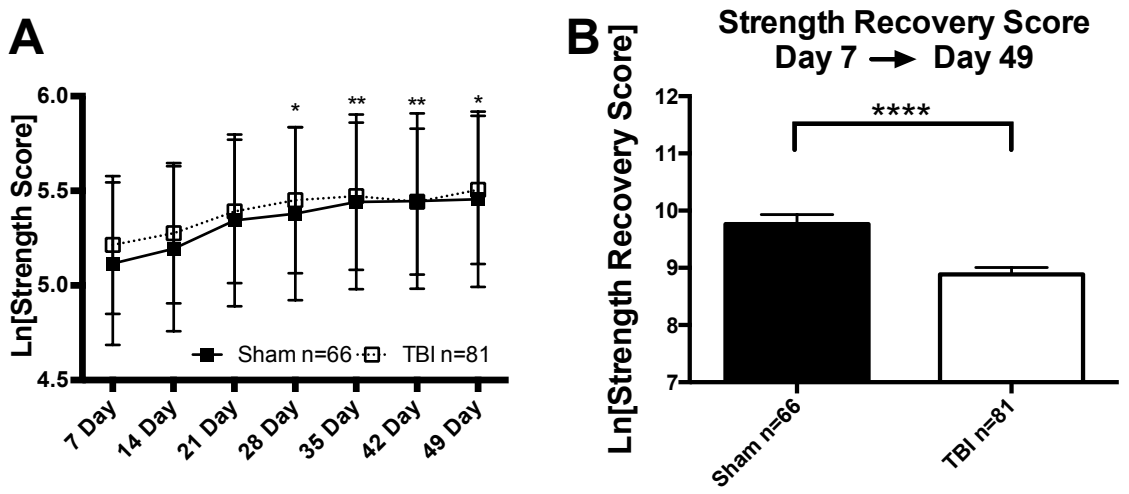


Figure 5. TBI Surgery Significantly Attenuated Grip Strength and Grip Strength Recovery. (A) Grip Strength is significantly attenuated by TBI compared to Sham ($F=64.618$, $p=0.000$). TBI surgery also significantly attenuated magnitude of grip strength score (Time x Surgery, $F=4.108$, $p=0.001$). Pairwise comparisons identified significant differences between Sham and TBI groups (B) Independent t-test identified significantly attenuated grip strength in TBI compared to Sham groups over 7 weeks TBI significantly attenuated overall grip strength recovery over 7 weeks of testing ($p<0.000$). *P≤0.1, **p≤0.01, ****p≤0.0001

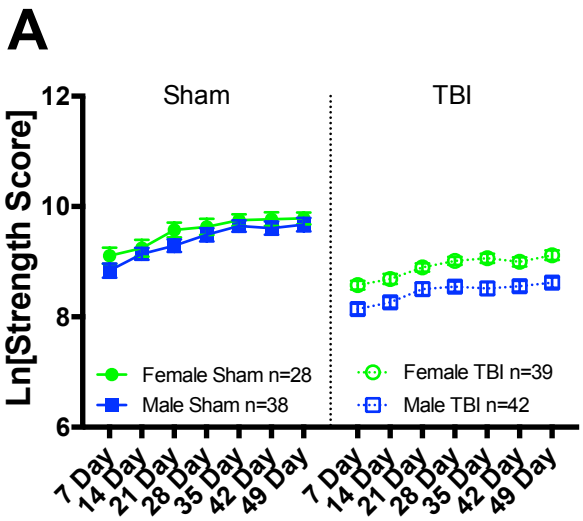


Figure 6. Sex Significantly Affected Grip Strength Recovery. (A) ANOVA identified an effect of sex ($F=11.156$, $p=0.001$) and an effect of sex x treatment x surgery ($F=3.048$, $p=0.083$). Post hoc comparison identified a significant difference between Female TBI and Male TBI ($p=0.002$). Pairwise comparisons did not identify any significant changes over time differences between males and females in each surgical group.

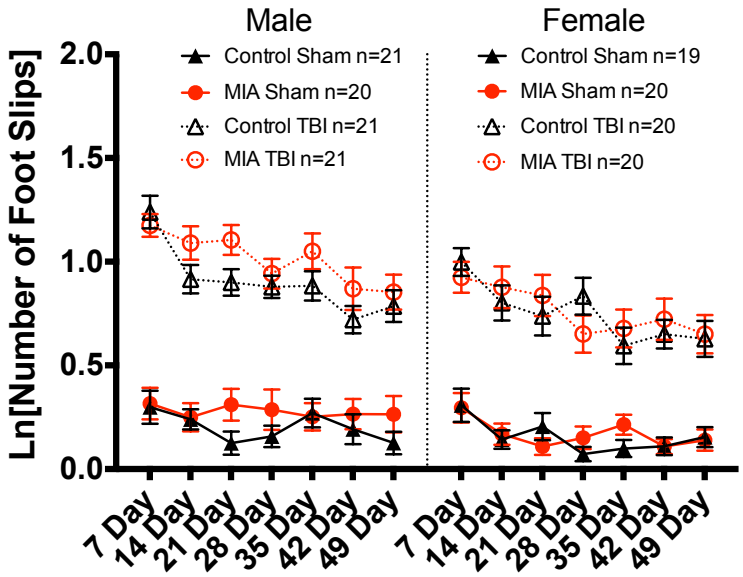


Figure 7: Overall Performance of All Experimental Groups on Balance Beam. All MIA treated and TBI groups are graphed separated by sex to show overall performance in Balance beam. Decrease in foot slips indicates recovery over time. TBI groups exhibit higher foot slips compared to sham groups (F=67.526, p=0.000).

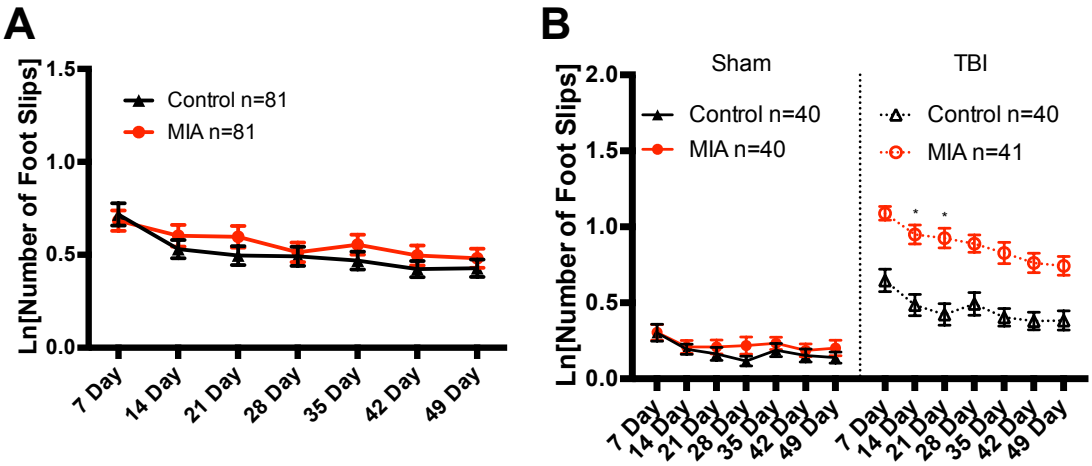


Figure 8: Comparison of MIA vs. Control offspring does not identify a main effect of treatment in Magnitude of Foot Slips during Recovery from Surgery. (A) 3-way ANOVA did not identify a main effect of treatment, or a significant time by MIA treatment interaction (F=2.227, p=0.138). Pairwise comparison did not identify significant differences between control and MIA at each recorded measure. (B) ANOVA identified significant effect of MIA treatment in TBI animals (F=2.041, p=0.048) but not in sham animals (F=0.332, p=0.993). Pairwise comparison identified significant differences at Day 14 (p=0.075) and Day 21 (p=0.048). *P≤0.1, **p≤0.01, ****p≤0.0001

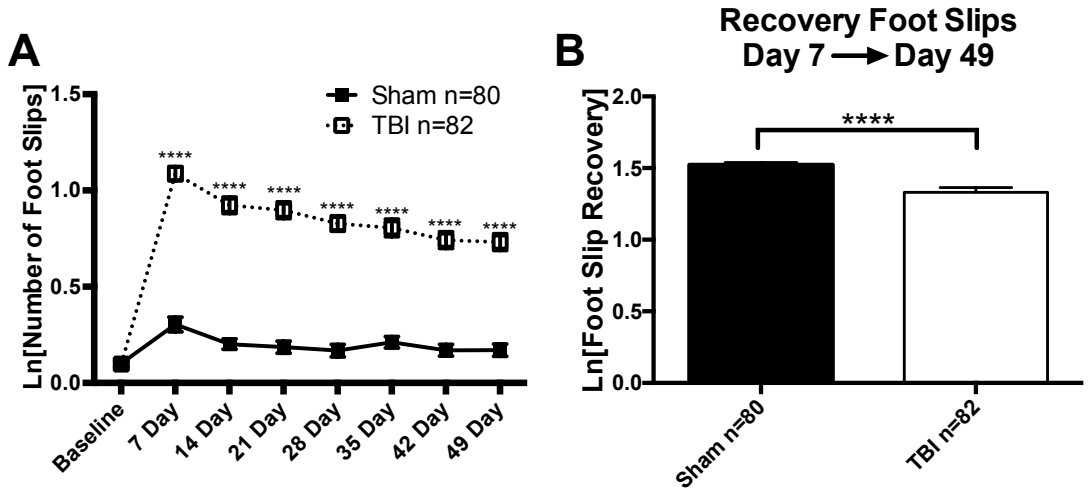


Figure 9: TBI Significantly Increased Balance Beam Foot Slips. (A) Foot slips are significantly increased by TBI compared to Sham (F=335.453, p=0.000). ANOVA identified a Time x Surgery interaction (F=35.677, p=0.000). Pairwise comparisons identified significant differences between TBI and sham groups from day 7 to day 49 (p<0.001). (B) The magnitude of recovery is greater in Sham compared to TBI treatment groups over 7 weeks of testing (p<0.0001). ****p≤0.0001

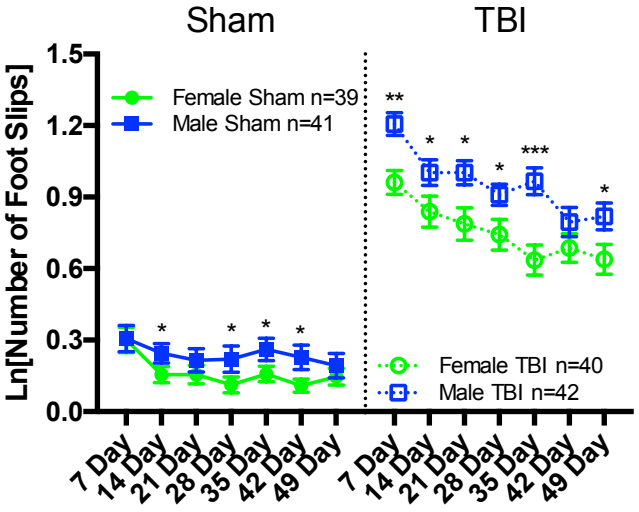


Figure 10: Males Displayed More Foot Slips than Females (A) ANOVA of foot slips identified a main effect of sex (F=16.509, p=0.000) and an interaction with time (F=2.217, p=0.031). It also identified a main effect of sex x surgery (F=3.541, p=0.062). Pairwise comparison identified significant sex differences between sham (p<0.1) and TBI groups (p<0.1). Post hoc comparisons identified differences between female TBI and male TBI (p=0.000) but not in female sham and male sham (p=0.803). *P≤0.1, **p≤0.01, ***p≤0.001

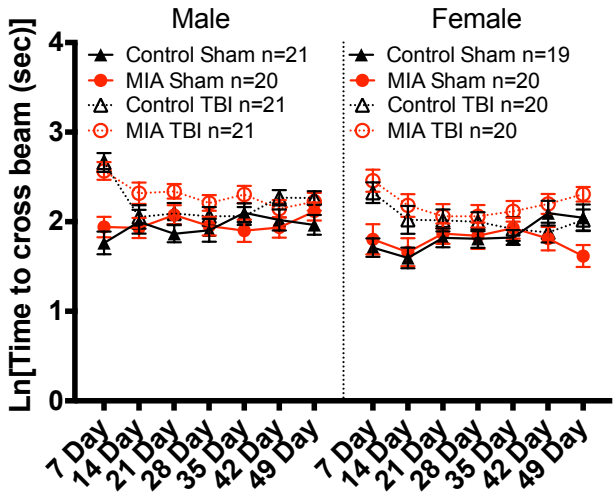


Figure 11: Overall Performance of All Experimental Groups on Latency to Cross Balance Beam. Individual surgical and MIA treatment groups are graphed separated by sex to show overall performance in Balance beam. Decrease in travel time indicates recovery over time. TBI groups in both sexes are slightly slower than sham groups in travel time (F=4.306, p=0.000).

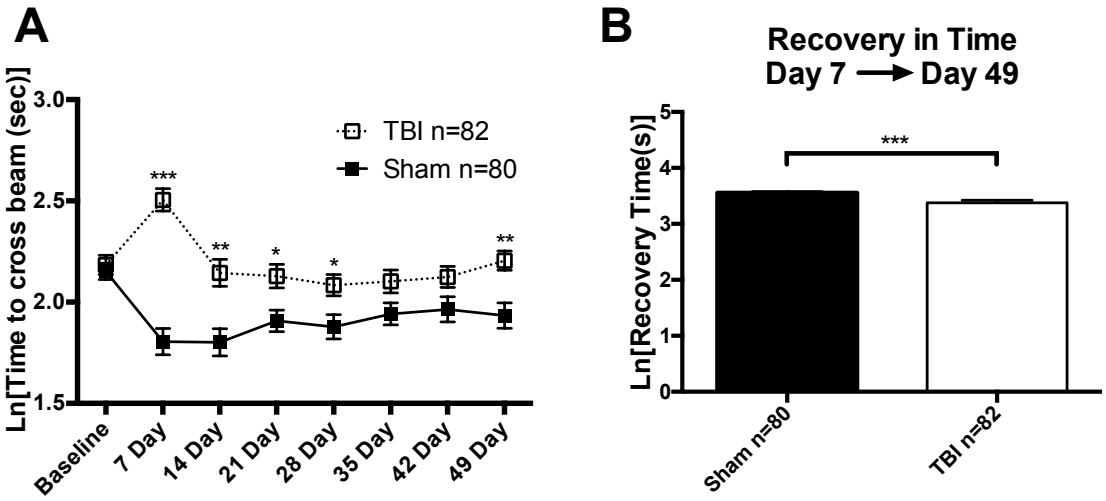


Figure 12: TBI Significantly Increased Latency to Cross Balance Beam. (A) Latency to cross the beam is significantly increased in TBI compared to Sham (F=33.238, p=0.000). ANOVA also identified a Time x Surgery interaction (F=7.885, p=0.000). Pairwise comparisons identified significantly increased time to cross the beam in TBI compared to Sham groups over 7 weeks with the greatest improvement seen in the first two weeks post-surgery (p<0.001), differences at week 3 and 7 (p<0.01), and differences at week 4 and 5 (p<0.05). (B) Improvement in Latency to cross the beam is significantly greater in TBI surgery compared to sham over 7 weeks of testing (p=0.0009). *P≤0.1, **p≤0.01, ***p≤0.001

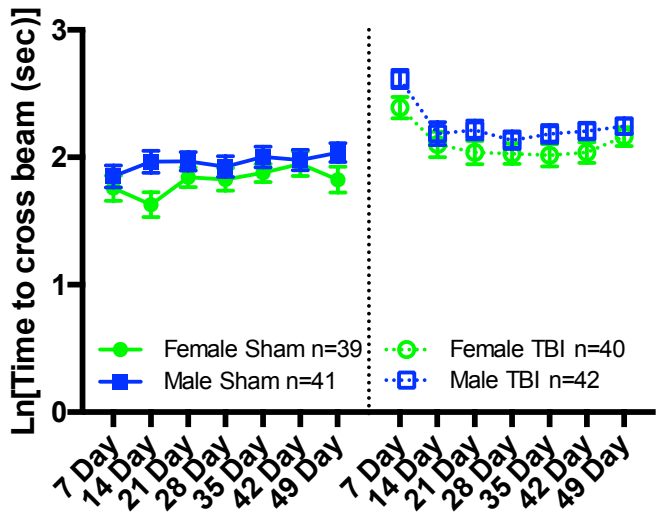


Figure 13: Males Have Significantly Attenuated Latency to Cross Beam. ANOVA identified a main effect of sex (F=9.005, p=0.003) and a 4-way interaction with time, sex, surgery, and MIA treatment (F=2.376, p=0.025). Pairwise comparisons did not identify any significant sex differences in each surgical group. Post hoc comparisons did not find any differences between groups (p>0.1).

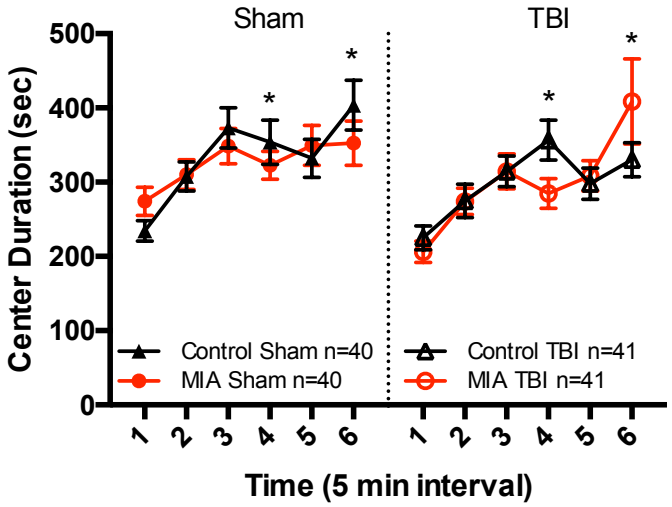


Figure 14. ANOVA Identified a Significant Time x Treatment Interaction in TBI Animals. (A) ANOVA identified a time x surgery x treatment interaction in center duration (F=2.571, p=0.04) and a significant interaction between time and treatment in TBI animals (F=2.483, p=0.073) but not in sham (F=1.473, p=0.204). Post hoc comparisons did not find any significant group differences (p=1.000). Pairwise comparisons identified significant treatment differences at block 4 and 6 in sham animals (p<0.05). *P≤0.1

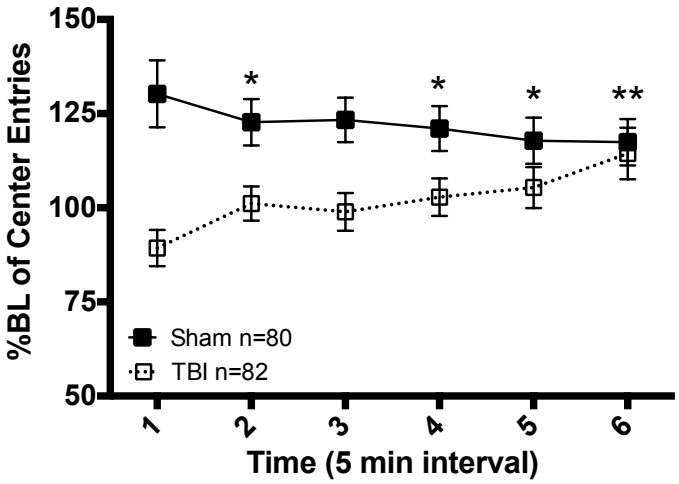


Figure 15. Effects of Surgery were Found Pre-Surgery in Center Entries. Post-surgical data calculated as a percent over pre-surgical data identified effects of surgery ($F=17.108$, $p=0.000$) along with a time x surgery interaction ($F=3.195$, $p=0.009$). Pairwise Comparisons identified significant differences between sham and TBI groups at second, fourth, and fifth intervals ($p<0.1$), and sixth interval ($p<0.01$). *P≤0.1, **P≤0.01

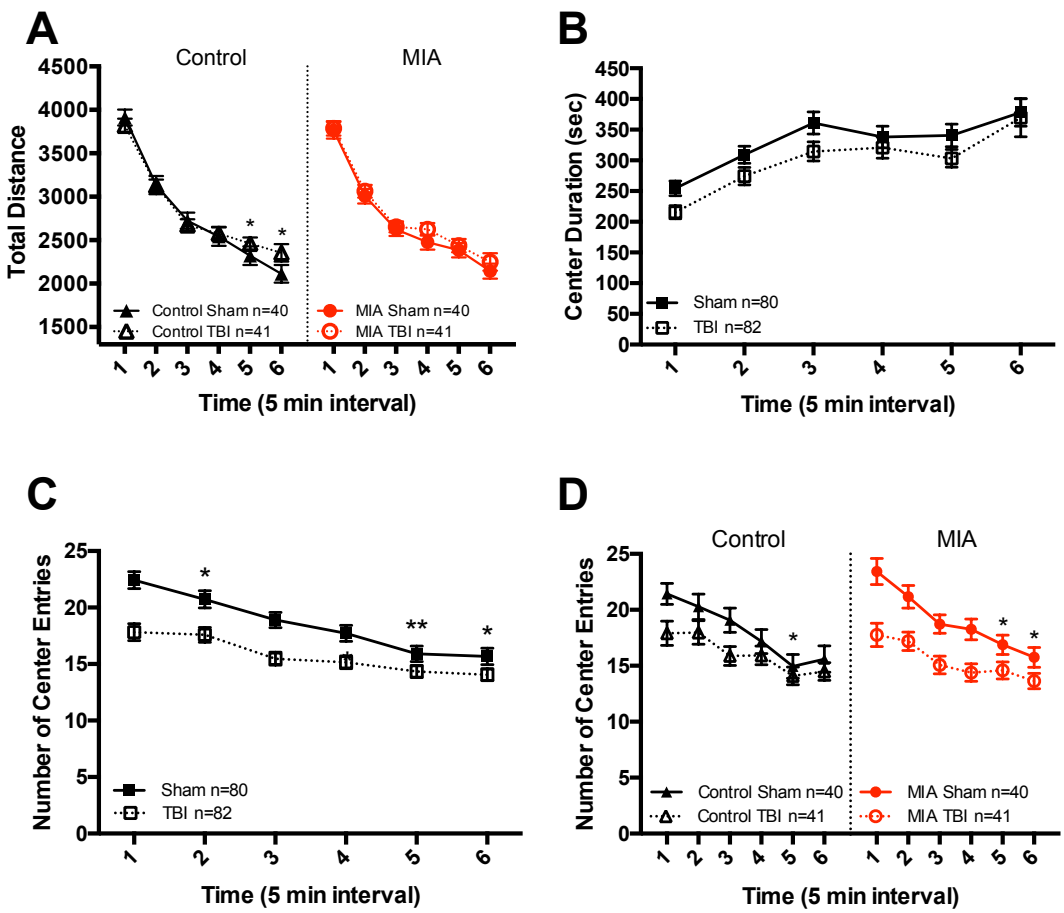


Figure 16. Mice Displayed Altered Behavior in Open Field After TBI. (A) ANOVA identified a time x surgery interaction in total distance traveled (F=2.530, p=0.039). Post hoc comparisons did not identify any significant differences between groups (p=1.000). Pairwise comparison identified significant effects of surgery in interval 5 and 6 (P<0.1). (B) ANOVA for center duration identified a main effect of surgery (F=3.335, p=0.070). (C) TBI significantly decreased number of center entries (F=14.742, p=0.000). ANOVA also identified a time x surgery interaction (F=3.172, p=0.012). Pairwise comparisons identified significant attenuated center entries in TBI compared to Sham in second, fourth, and sixth intervals (p<0.1), and fifth interval (p<0.01). (D) ANOVA of center entries identified a main effect of surgery in both control and MIA animals (F=3.217, p=0.077) and (F=15.018, p=0.000); however, ANOVA only identified a time by surgery interaction in the MIA group (F=1.961, p=0.096) but not in control (F=1.556, p=0.185). *P≤0.1, **P≤0.01

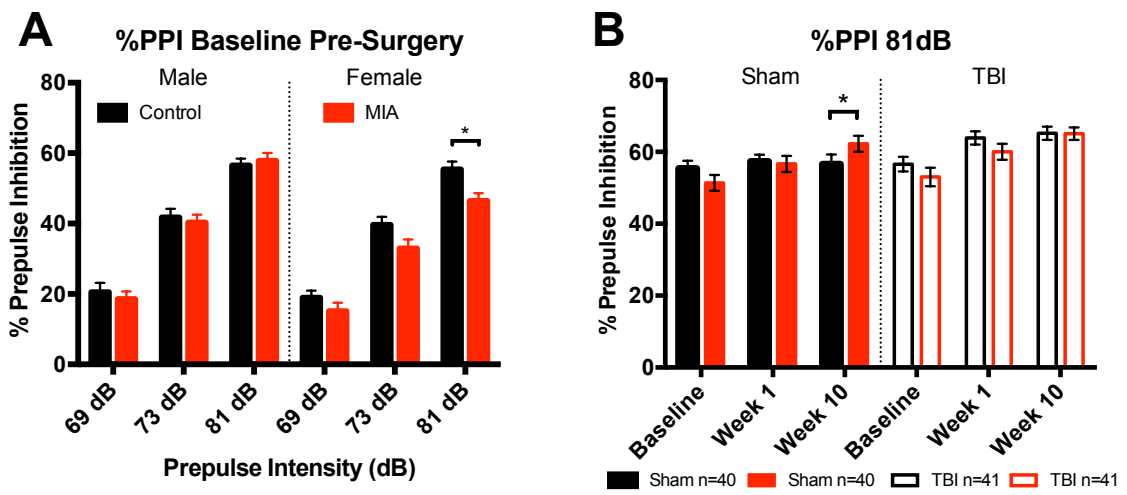


Figure 17. MIA Treatment Interaction and Effects on Prepulse Inhibition of Startle. (A) ANOVA of baseline values identified an effect of sex ($F=11.874$, $p=0.001$). ANOVA also identified an effect of treatment x sex effect ($F=3.151$, $p=0.078$). Independent t-tests identified significant deficit in PPI in Female MIA offspring at the 81db prepulse ($p=0.0056$). (B) 3-way ANOVA of 81dB prepulse identified an interaction between time and treatment ($F=3.760$, $p=0.027$) and analysis shows an effect of MIA treatment in sham ($F=3.902$, $p=0.026$) but not in TBI animals ($F=0.679$, $p=0.502$). Pairwise comparison identified an effect of treatment at week 10 in sham animals ($p<0.05$). *$P \leq 0.1$

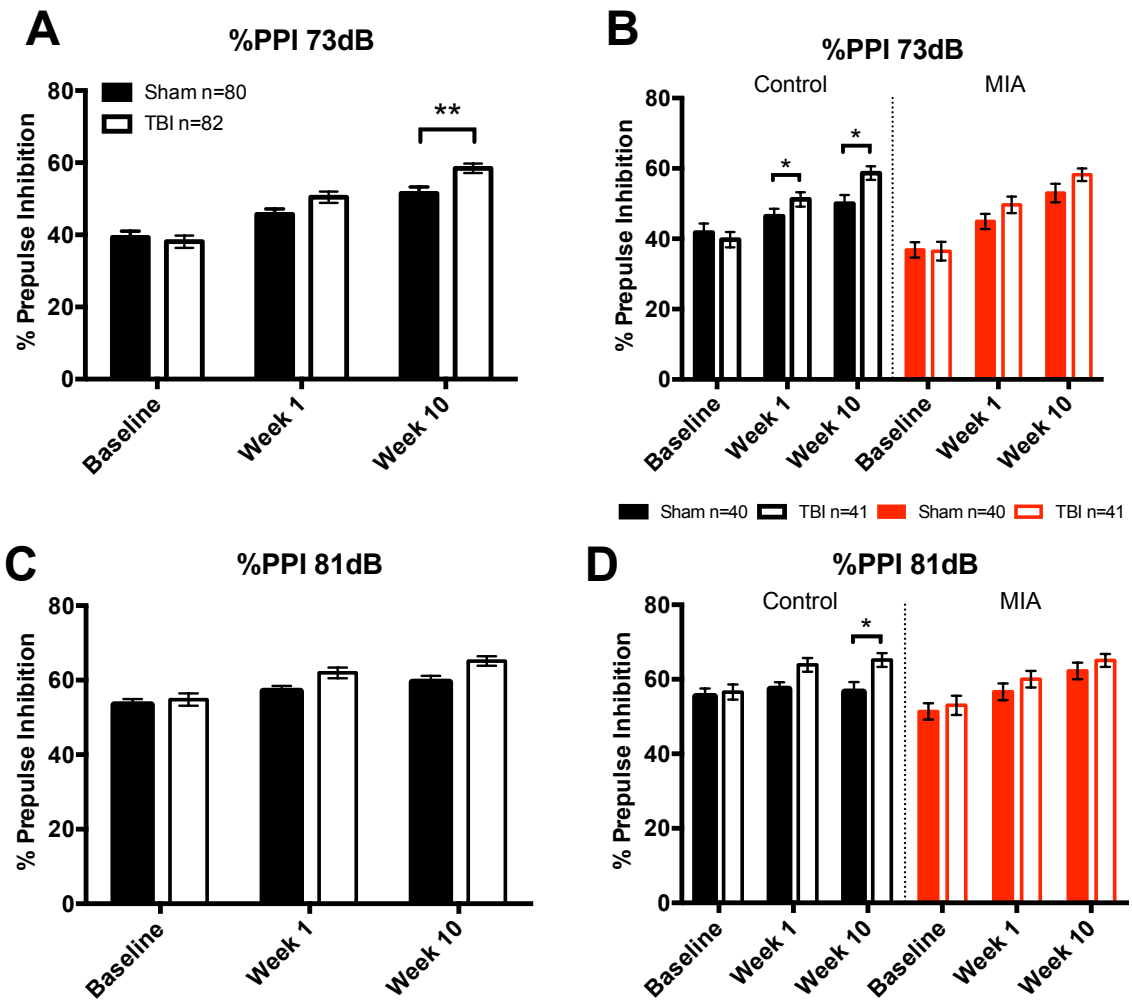


Figure 18. TBI Significantly Increases Prepulse Inhibition of Startle. (A) ANOVA at 73dB prepulse identified a time x surgery effect ($F=4.637$, $p=0.011$) and a main effect of surgery ($F=4.669$, $p=0.032$). Pairwise comparisons identified significantly increased PPI in TBI compared to Sham at week 10 ($p<0.01$). (B) ANOVA identified a trend towards a main effect of surgery in control animals ($F=2.739$, $p=0.102$) and in MIA ($F=1.956$, $p=0.166$). ANOVA also identified an interaction of time and surgery in control animals ($F=4.224$, $p=0.016$). Pairwise comparison identified significant effects of surgery at week 1 and week 10 in Control mice ($p<0.05$). (C) ANOVA at 81dB prepulse identified a significant main effect of surgery ($F=6.430$, $p=0.012$). Pairwise comparison did not identify any significant effects of surgery at either week 1 or week 10. (D) ANOVA identified main effects of surgery in control animals ($F=6.314$, $p=0.014$) but not in MIA ($F=1.267$, $p=0.264$). ANOVA also identified an interaction of time and surgery ($F=2.385$, $p=0.098$). Pairwise comparison identified significant effects of surgery at week 10 ($p<0.1$). *P≤0.1, **P≤0.01

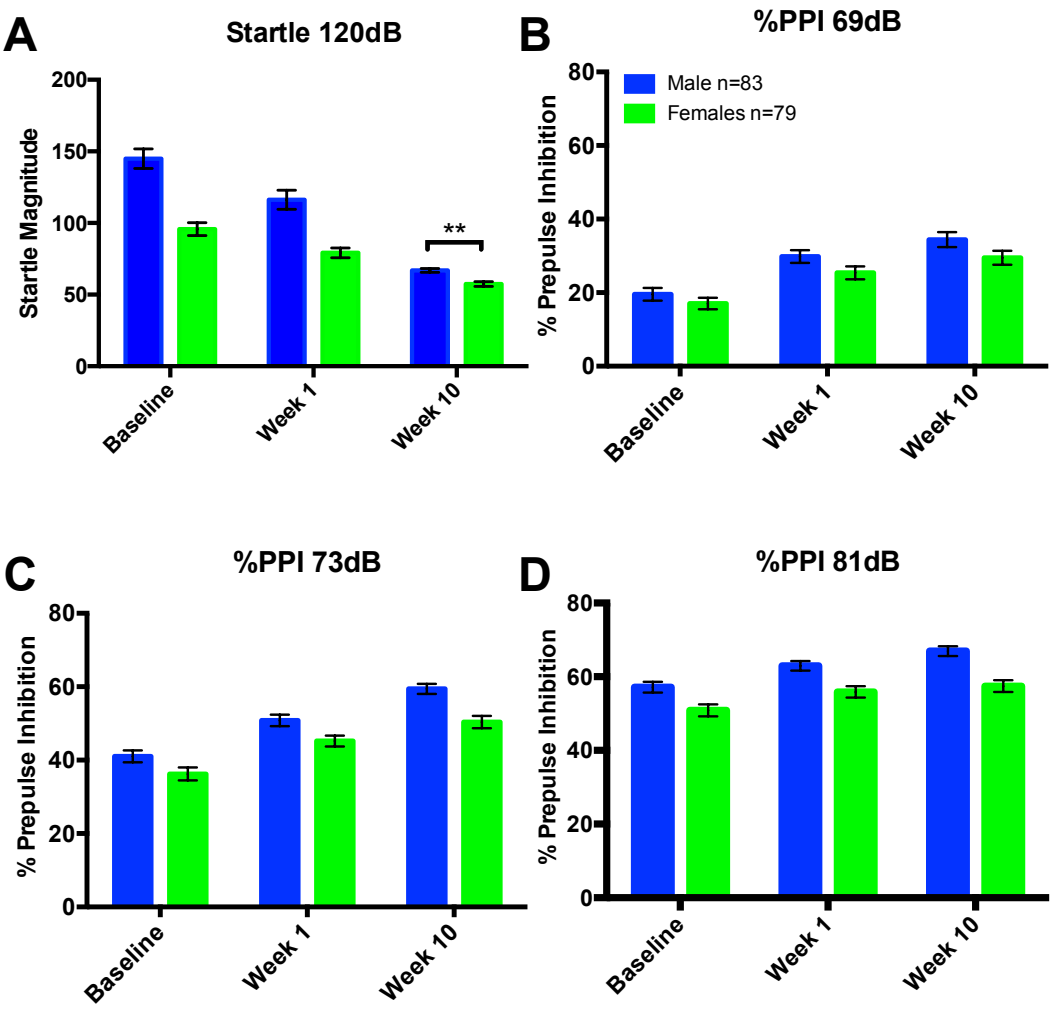


Figure 19. Both Startle Magnitude and PPI are Inhibited in Females. (A) Females displayed significantly lower startle magnitude compared to males upon startle stimulus (F=45.406, p=0.000). ANOVA also identified a time x sex interaction (F=5.571, p=0.004). Pairwise comparisons identified significantly lower startle response in females compared to males at baseline and week 10 (p=0.002). (B, C, D) ANOVA identified a significant effect of sex on %PPI inhibition at 69dB (F=4.817, p=0.030), 73dB (F=17.622, p=0.000), and 81dB (F=26.733, p=0.000). At the 73dB prepulse, ANOVA identified a time x sex x surgery effect (F=3.397, p=0.035). At the 81dB prepulse, ANOVA identified an effect of sex x surgery (F=3.328, p=0.070). Pairwise comparisons at 69dB, 73dB, and 81dB prepulses did not find any significant differences between females and males over 3 time points. **p≤0.01

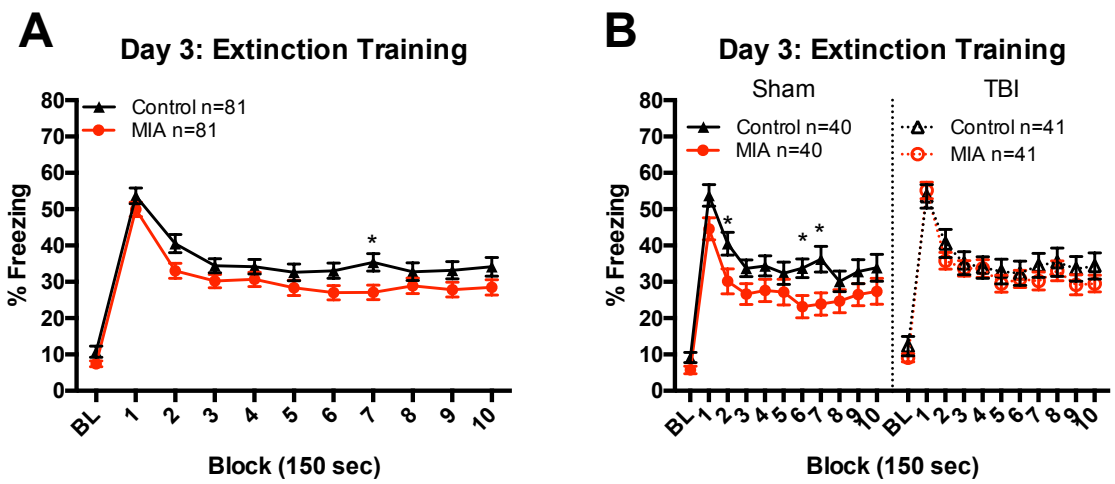


Figure 20. MIA Sham Animals Display Lower Freezing than Control Sham. (A) ANOVA of time, surgery, and MIA treatment on day 3: Extinction Training identified a significant effect of surgery (F=4.698, p=0.032). Pairwise comparison identified significant effect of MIA treatment at block 7 (p<0.1). (B) ANOVA identified significant effects of MIA treatment in sham animals (F=4.972, p=0.029) but not in TBI animals (F=0.648, p=0.423). Pairwise comparisons identified significant effects of MIA treatment at block 2, 6, and 7 (p<0.1). Post hoc comparisons did not identify any significant group differences (p=1.000)
*p≤0.1

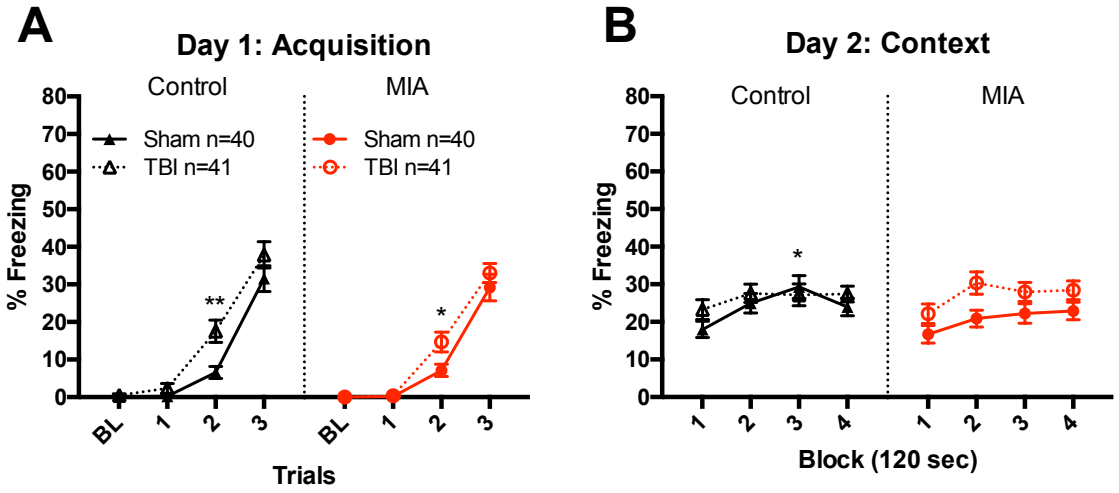


Figure 21. TBI Animals Display Higher Freezing than Sham Animals in Fear Conditioning. (A) ANOVA of time, surgery, and MIA treatment on day 1: Acquisition identified a main effect of surgery (F=9.839 p=0.002). It also identified an interaction between time and surgery (F=5.046, p=0.009). Pairwise comparison identified a significant effect of surgery at trial 2 in control animals (p<0.01) and MIA animals (p<0.1). Post hoc comparisons identified a group differences between control sham and control TBI (p=0.035). Interestingly, MIA sham and MIA TBI was not found to be significant (p=0.621). (B) ANOVA of time, surgery, and MIA treatment on day 2: Context identified a main effect of surgery (F=4.531, p=0.035). Pairwise comparison identified a significant surgery effect in control animals at block 3 (p<0.1).
*p≤0.1, **p≤0.01

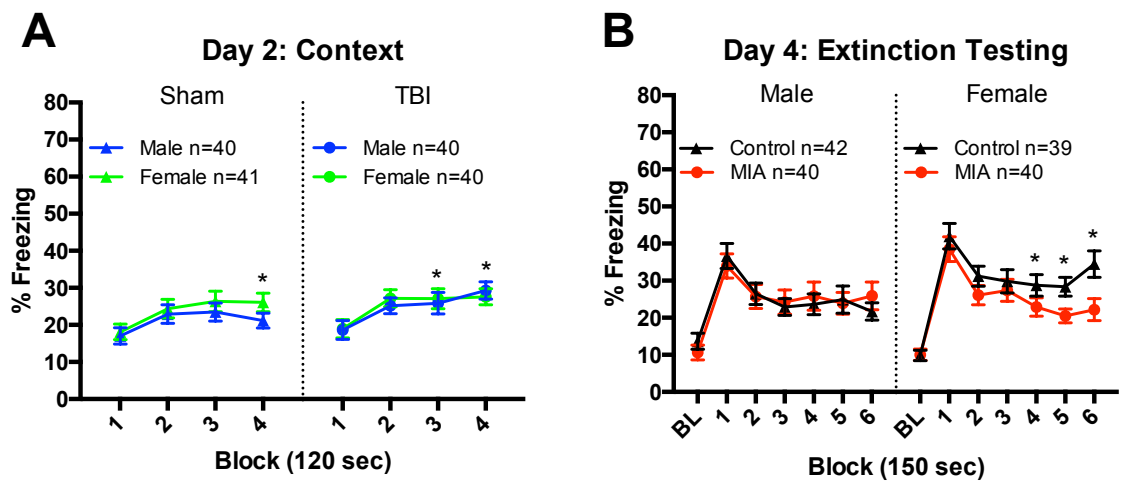


Figure 22. Significant Effects of Sex Observed in Fear Conditioning. (A) 4-way ANOVA of time, sex, MIA treatment, and surgery at Day 2: Context identified an interaction of time and sex ($F=3.273$, $p=0.021$). ANOVA also found a sex x surgery effect ($F=3.762$, $p=0.054$). Pairwise comparisons identified a significant effect of sex at block 4 in sham animals ($p<0.1$) and block 3 and 4 in TBI animals ($p<0.1$). Post hoc comparisons identified group differences between male sham and male TBI ($p=0.027$) but no differences were found in females ($p=0.1000$). (B) 4-way ANOVA of time, sex, MIA treatment, and surgery at Day 4: Extinction Testing identified a time x sex x treatment effect ($F=3.712$, $p=0.001$). Pairwise comparison identified a significant effect of treatment in females at block 4, 5, and 6 ($p<0.1$).

Females

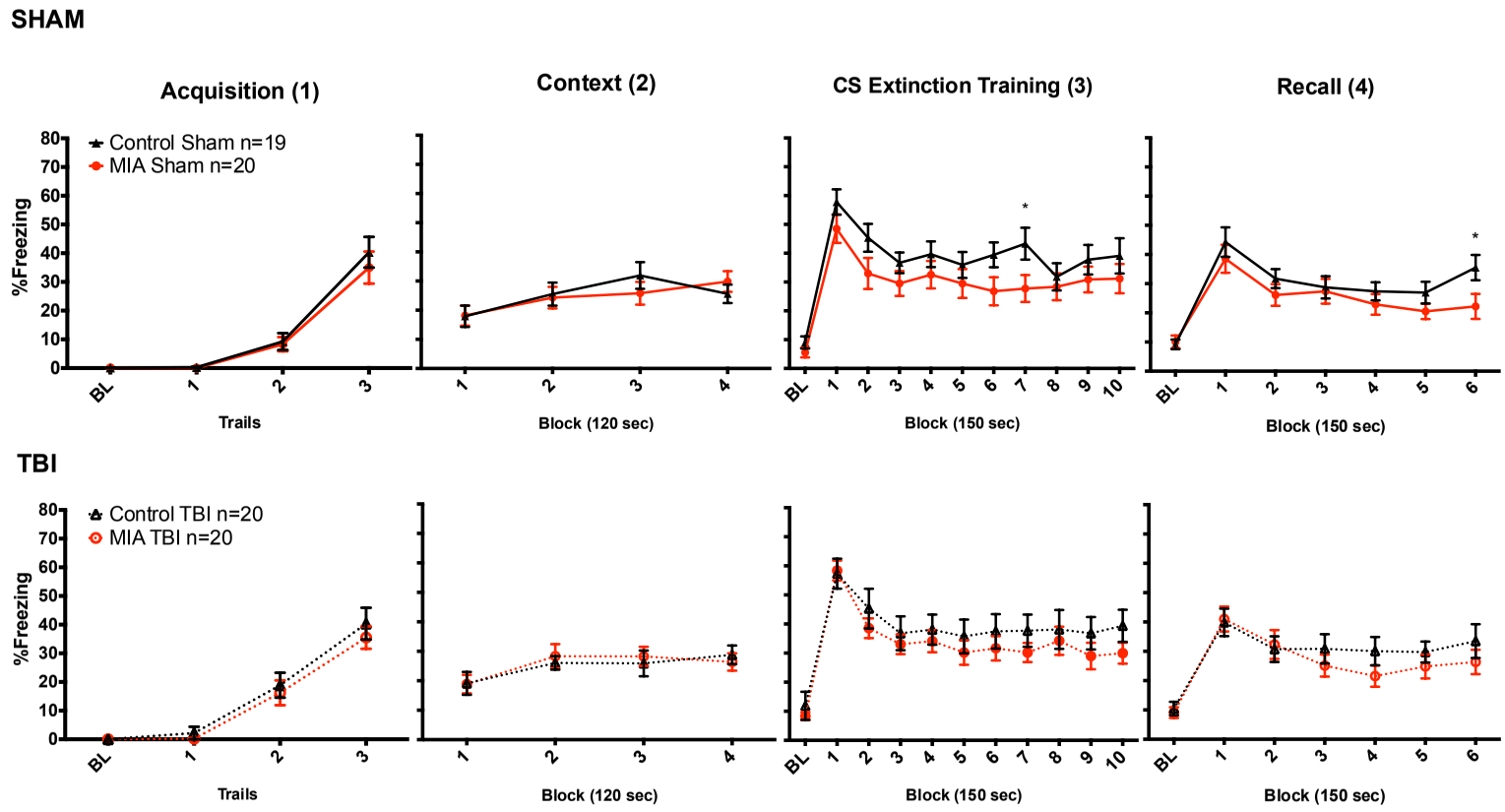


Figure 23. Overall Performance of Female Experimental Groups on Fear Conditioning. Panel 1 shows acquisition in which animals were presented a tone associated with a foot shock. Panel 2 shows context in which animals were placed into the same box without any tone or foot shock. Panel 3 shows extinction training in which the animal is presented with a new context with tones. Panel 4 shows extinction testing in which the animal is presented with the same contextual cues as day 3 along with tones. Pairwise comparisons of day 3 identified a significant effect of treatment in sham animals on block 7 ($p<0.1$). Pairwise comparisons of day 4 identified significant effects of treatment in sham animals on block 6 ($p<0.05$). *P≤0.1

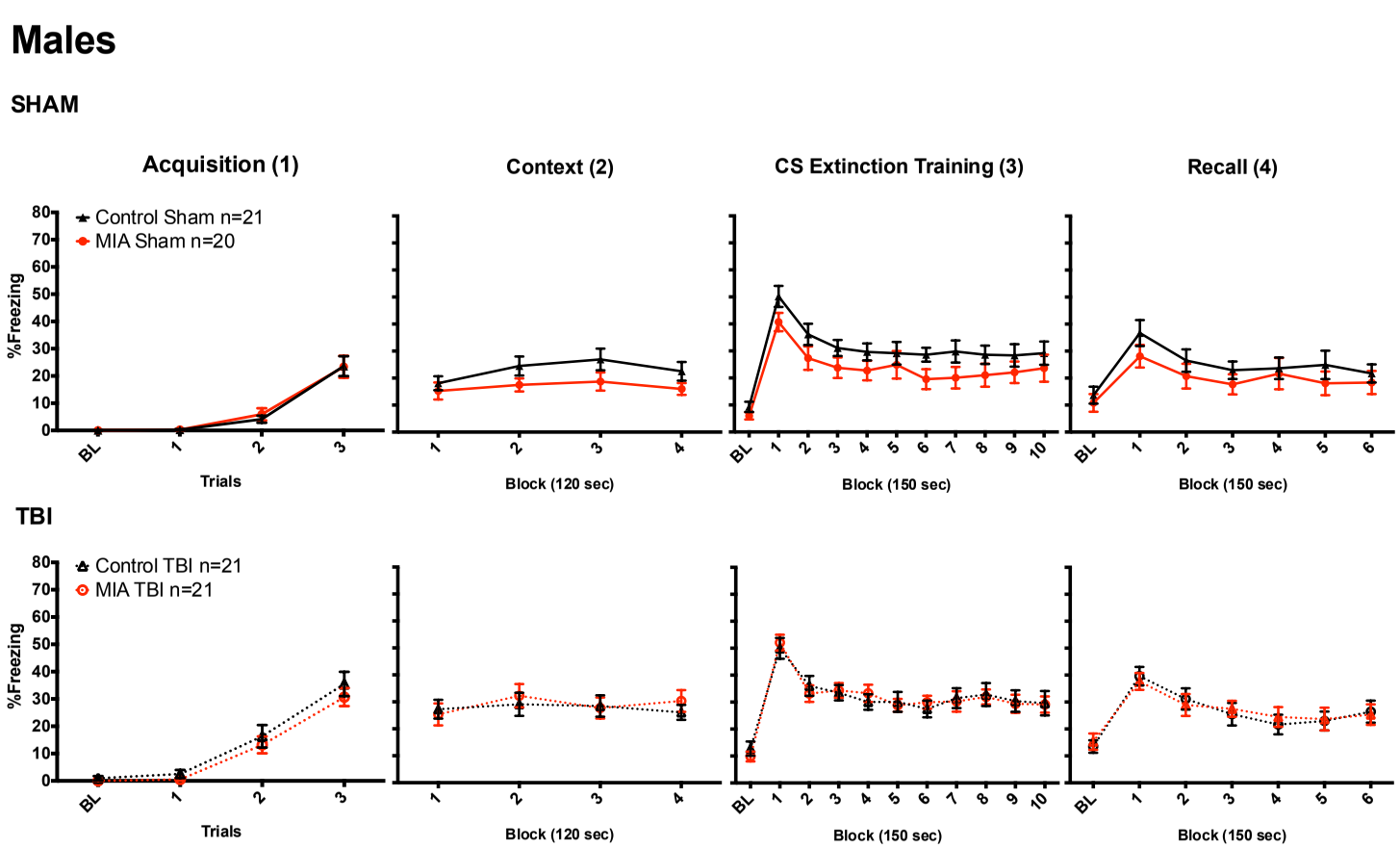


Figure 24. Overall Performance of Male Experimental Groups on Fear Conditioning. Panel 1 shows acquisition in which animals were presented a tone associated with a foot shock. Panel 2 shows context in which animals were placed into the same box without any tone or foot shock. Panel 3 shows extinction training in which the animal is presented with a new context with tones. Panel 4 shows extinction testing in which the animal is presented with the same contextual cues as day 3 along with tones. Pairwise comparisons did not identify any significant effects of treatment in each day.

Male

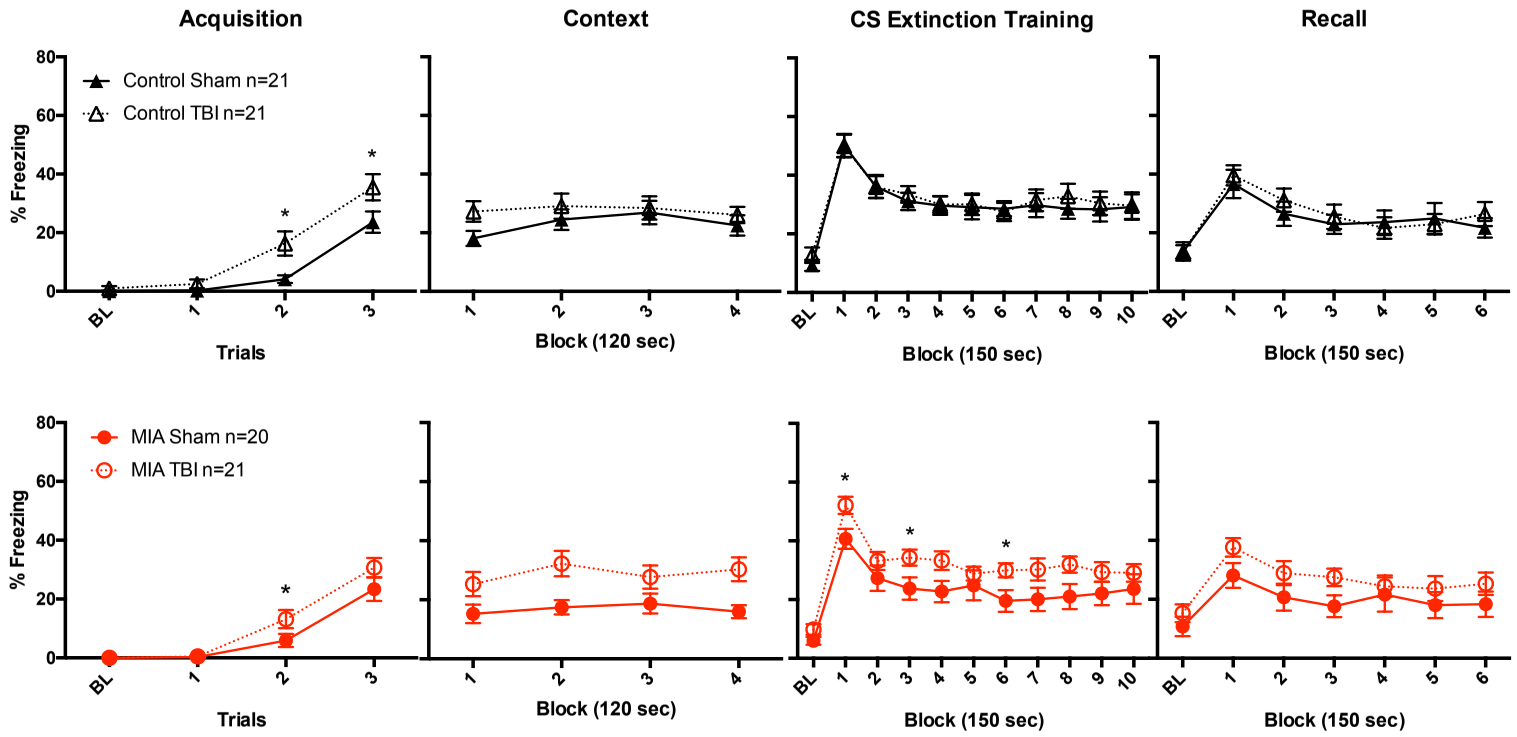


Figure 25. TBI Significantly Increased Overall Freezing in Males on Fear Conditioning. (Panel 1) Control TBI displayed increased freezing on trial 2 and 3 ($p<0.1$). MIA TBI displayed increased freezing on trial 2 ($p<0.1$). (Panel 3) MIA TBI displayed increased freezing on block 1, 3, and 6 ($p<0.1$). *P≤0.1

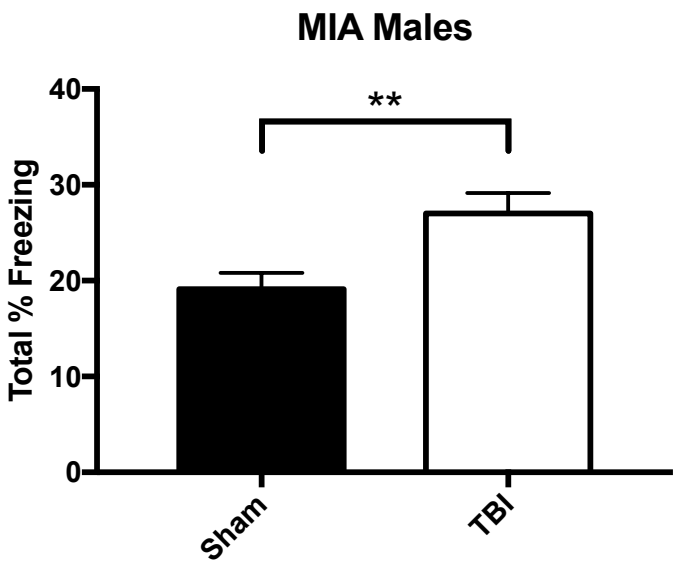


Figure 26. TBI Significantly Increased Freezing in MIA Males. Compared to MIA/Sham, freezing was significantly increased in MIA/TBI animals through all 4 days of testing ($p=0.0059$). **$p \leq 0.01$,

Table

Table 1. Areas of Injury from Controlled Cortical Impact. Based on the site and depth of the CCI injury, different areas of the brain may be affected.

AuD	Secondary Auditory cortex
CA1, CA2	Field in Hippocampus
Cg1, Cg2	Cingulate Cortex area 1/2
FC	Fasciola Cinereum
IG	Indusium Griseum
LMOL	Lacunosum moleculare layer of the Hippocampus
LPta	Lateral Parietal association cortex
LSD	Lateral Septal nucleus, dorsal part
M1, M2	Primary and Secondary Motor cortex
MPta	Medial Parietal Association cortex
Or	Oriens layer of the Hippocampus
Py	Pyramidal tract
Rad	Stratum Radiatum of the Hippocampus
RSA, RSG	Retrosplenial Agranular cortex, Granular cortex
S	Subicum
S1BF	Primary Somatosensory cortex, barrel field
S1DZ	Primary Somatosensory cortex, dysgranular region
S1FL	Primary Somatosensory cortex, forelimb region
S1HL	Primary Somatosensory cortex, hindlimb region
S1ShNc	Primary Somatosensory cortex, shoulder/neck region
S1Tr	Primary Somatosensory cortex, trunk region
S1ULp	Primary Somatosensory cortex, upper lip region
SLu	Stratum Lucidum, hippocampus
V1	Primary Visual cortex
V2L	Secondary Visual cortex, lateral area
V2ML	Secondary Visual cortex, mediolateral area
V2MM	Secondary Visual cortex, mediomedial area

Table 2. ANOVA Results Table for Inverted Grid Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Inverted Grid				
		3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=68.713 p=0.000	F=55.526 p=0.000	F=20.905 p=0.000	F=44.697 p=0.000	F=27.442 p=0.000
	Time*Tx	F=2.462 p=0.034	F=2.606 p=0.037	NS		
	Time*Surgery	F=4.108 p=0.001			F=4.600 p=0.001	NS
	Time*Tx*surgery	NS				
3-Way ANOVA Between Subject	Surgery	F=64.617 p=0.000			F=25.538 p=0.000	F=41.064 p=0.000
	Tx	NS	NS	NS		
	Tx*Surgery	NS				
4-Way ANOVA Within Subject	Time*Sex	NS				
	Time*Sex*Tx					
	Time*Sex*Surgery					
	Time*Sex*Tx*Surgery					
4-Way ANOVA Between Subject	Sex	F=11.156 p=0.001				
	Sex*Tx					
	Sex*Surgery					
	Sex*Tx*Surgery	F=3.048 p=0.083				

Table 3. ANOVA Results Table for Balance Beam Assays Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Balance Beam Foot Slips					Balance Beam Time				
		3-Way ANOVA	Sham	TBI	Control	MIA	3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=67.526 p=0.000	F=4.694 p=0.000	F=82.791 p=0.000	F=40.973 p=0.000	F=29.786 p=0.000	F=4.306 p=0.000	F=4.276 p=0.001	F=8.446 p=0.000	F=3.292 p=0.003	F=1.808 p=0.098
	Time*Tx	NS	NS	F=2.041 p=0.048			NS	NS	NS		
	Time*Surgery	F=35.677 p=0.000			F=20.228 p=0.000	F=17.454 p=0.000	F=7.885 p=0.000			F=5.685 p=0.000	F=3.776 p=0.001
	Time*Tx*Surgery	NS					NS				
3-Way ANOVA Between Subject	Surgery	F=335.453 p=0.000			F=181.196 p=0.000	F=156.370 p=0.000	F=33.238 p=0.000			F=10.227 p=0.002	F=24.765 p=0.000
	Tx	NS	NS	NS			NS	NS	NS		
	Tx*Surgery	NS					NS				
4-Way ANOVA Within Subject	Time*Sex	F=2.217 p=0.031					NS				
	Time*Sex*Tx										
	Time*Sex*Surgery										
	Time*Sex*Tx*Surgery						F=2.376 p=0.025				
4-Way ANOVA Between Subject	Sex	F=16.509 p=0.000					F=9.005 p=0.003				
	Sex*Tx										
	Sex*Surgery	F=3.541 p=0.062									
	Sex*Tx*Surgery										

Table 4. ANOVA Results Table for Travel Distance and Crossovers from The Open Field Assay. Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Open Field Distance					Open Field Crossover Entries				
		3-Way ANOVA	Sham	TBI	Control	MIA	3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=567.674 p=0.000	F=290.447 p=0.000	F=277.577 p=0.000	F=307.797 p=0.000	F=261.819 p=0.000	F=416.216 p=0.000	F=203.409 p=0.000	F=214.068 p=0.000	F=229.151 p=0.000	F=188.188 p=0.000
	Time*Tx	NS	NS	NS			NS	NS	NS		
	Time*Surgery	F=2.530 p=0.039			F=3.352 p=0.012	NS	NS			NS	NS
	Time*Tx*Surgery	NS					NS				
3-Way ANOVA Between	Surgery	NS			NS	NS	NS			NS	NS
	Tx	NS	NS	NS			NS	NS	NS		
	Tx*Surgery	NS					NS				
4-Way ANOVA Within	Time*Sex	NS					NS				
	Time*Sex*Tx										
	Time*Sex*Surgery										
	Time*Sex*Tx*Surgery										
4-Way ANOVA Between Subject	Sex	F=4.570 p=0.034					NS				
	Sex*Tx										
	Sex*Surgery										
	Sex*Tx*Surgery										

Table 5. ANOVA Results Table for Center Time and Entries from The Open Field Assay. Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Open Field Center Time					Open Field Center Entries				
		3-Way ANOVA	Sham	TBI	Control	MIA	3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=20.690 p=0.000	F=9.932 p=0.000	F=11.166 p=0.000	F=14.606 p=0.000	F=8.629 p=0.000	F=42.941 p=0.000	F=29.459 p=0.000	F=14.293 p=0.000	F=21.029 p=0.000	F=23.141 p=0.000
	Time*Tx	NS	NS	F=2.483 p=0.073			NS	NS	NS		
	Time*Surgery	NS			NS	NS	F=3.172 p=0.012			NS	F=1.961 p=0.096
	Time*Tx*Surgery	F=2.571 p=0.047					NS				
3-Way ANOVA Between Subject	Surgery	F=3.335 p=0.070			NS	NS	F=14.742 p=0.000			F=3.217 p=0.077	F=15.018 p=0.000
	Tx	NS	NS	NS			NS	NS	NS		
	Tx*Surgery	NS					NS				
4-Way ANOVA Within Subject	Time*Sex	NS					NS				
	Time*Sex*Tx	F=2.501 p=0.051									
	Time*Sex*Surgery						NS				
	Time*Sex*Tx*Surgery										
4-Way ANOVA Between Subject	Sex						F=3.341 p=0.069				
	Sex*Tx	F=5.783 p=0.017									
	Sex*Surgery										
	Sex*Tx*Surgery										

Table 6. ANOVA Results Table for Baseline Startle and %PPI at 69dB from Prepulse Inhibition of Startle Assay. Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Baseline Startle at 120dB					%PPI 69dB				
		3-Way ANOVA	Sham	TBI	Control	MIA	3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=62.806 p=0.000	F=34.927 p=0.000	F=28.523 p=0.000	F=34.115 p=0.000	F=28.774 p=0.000	F=40.820 p=0.000	F=16.394 p=0.000	F=24.888 p=0.000	F=13.222 p=0.000	F=30.432 p=0.000
	Time*Tx	NS	NS	NS			F=2.254 p=0.107	NS	NS		
	Time*Surgery	NS			NS	NS	NS			NS	NS
	Time*Tx*Surgery	NS					NS				
3-Way ANOVA Between	Surgery	NS			NS	NS	NS			NS	NS
	Tx	NS	NS	NS			NS	NS	NS		
	Tx*Surgery	NS					NS				
4-Way ANOVA Within Subject	Time*Sex	F=5.571 p=0.004					NS				
	Time*Sex*Tx										
	Time*Sex*Surgery										
	Time*Sex*Tx*Surgery										
4-Way ANOVA Between Subject	Sex	F=45.406 p=0.000					F=4.817 p=0.030				
	Sex*Tx										
	Sex*Surgery										
	Sex*Tx*Surgery										

Table 7. ANOVA Results Table for %PPI at 73dB and 81dB from Prepulse Inhibition of Startle Assay. Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		%PPI 73dB					%PPI 81dB				
		ALL	Sham	TBI	Control	MIA	ALL	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=69.468 p=0.000	F=18.479 p=0.000	F=57.429 p=0.000	F=26.763 p=0.000	F=43.090 p=0.000	F=22.063 p=0.000	F=5.963 p=0.004	F=17.648 p=0.000	F=4.955 p=0.009	F=20.498 p=0.000
	Time*Tx	NS	NS	NS			F=3.760 p=0.027	F=3.902 p=0.026	NS		
	Time*Surgery	F=4.637 p=0.011			F=4.224 p=0.016	NS	NS			F=2.385 p=0.098	NS
	Time*Tx*Surgery	NS					NS				
3-Way ANOVA Between Subject	Surgery	F=4.669 p=0.032			F=2.739 p=0.102	NS	F=6.430 p=0.012			F=6.314 p=0.014	NS
	Tx	NS	NS	NS			NS	NS	NS		
	Tx*Surgery										
4-Way ANOVA Within Subject	Time*Sex	NS					NS				
	Time*Sex*Tx										
	Time*Sex*Surgery	F=3.397 p=0.035					F=2.325 p=0.102				
	Time*Sex*Tx*Surgery										
4-Way ANOVA Between Subject	Sex	F=17.622 p=0.000					F=26.733 p=0.000				
	Sex*Tx										
	Sex*Surgery						F=3.328 p=0.070				
	Sex*Tx*Surgery										

Table 8. ANOVA Results Table for Day 1 and 2 from Fear Conditioning Assay Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Fear Conditioning Day 1					Fear Conditioning Day 2				
		3-Way ANOVA	Sham	TBI	Control	MIA	3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=275.402 p=0.000	F=132.001 p=0.000	F=147.837 p=0.000	F=140.078 p=0.000	F=135.605 p=0.000	F=13.423 p=0.000	F=10.286 p=0.000	F=4.994 p=0.002	F=7.293 p=0.000	F=6.745 p=0.000
	Time*Tx	NS	NS	NS			NS	NS	NS		
	Time*Surgery	F=5.046 p=0.009			F=3.067 p=0.055	NS	NS			NS	NS
	Time*Tx*Surgery	NS					NS				
3-Way ANOVA Between Subject	Surgery	F=9.839 p=0.002			F=6.341 p=0.014	F=3.505 p=0.065	F=4.531 p=0.035			NS	F=4.938 p=0.029
	Tx	NS					NS				
	Tx*Surgery	NS					NS				
4-Way ANOVA Within Subject	Time*Sex	F=6.373 p=0.003					F=3.273 p=0.021				
	Time*Sex*Tx										
	Time*Sex*Surgery										
	Time*Sex*Tx*Surgery										
4-Way ANOVA Between Subject	Sex	F=6.196 p=0.014									
	Sex*Tx										
	Sex*Surgery						F=3.762 p=0.054				
	Sex*Tx*Surgery										

Table 9. ANOVA Results Table for Day 3 and 4 from Fear Conditioning Assay. Data was initially analyzed through 3-Way Repeated Measures ANOVA. Data was then split separately by surgery (Sham and TBI columns) and treatment (Control and MIA columns) for further analysis. 4-Way Repeated Measures ANOVA was then used to analyze for any affects of sex. NS = not significant

		Fear Conditioning Day 3					Fear Conditioning Day 4				
		3-Way ANOVA	Sham	TBI	Control	MIA	3-Way ANOVA	Sham	TBI	Control	MIA
3-Way ANOVA Within Subject	Time	F=108.712 p=0.000	F=51.150 p=0.000	F=58.527 p=0.000	F=47.966 p=0.000	F=64.311 p=0.000	F=83.758 p=0.000	F=39.727 p=0.000	F=44.634 p=0.000	F=45.839 p=0.000	F=38.678 p=0.000
	Time*Tx	NS	NS	NS			NS	NS	NS		
	Time*Surgery	NS			NS	NS	NS			NS	NS
	Time*Tx*Surgery	NS					NS				
3-Way ANOVA Between Subject	Surgery	NS			NS	F=3.372 p=0.070	NS			NS	NS
	Tx	F=4.698 p=0.032	F=4.972 p=0.029	NS			NS				
	Tx*Surgery	NS					NS				
4-Way ANOVA Within Subject	Time*Sex	F=5.646 p=0.019									
	Time*Sex*Tx						F=3.712 p=0.001				
	Time*Sex*Surgery										
	Time*Sex*Tx*Surgery										
4-Way ANOVA Between Subject	Sex	F=5.016 p=0.027									
	Sex*Tx										
	Sex*Surgery										
	Sex*Tx*Surgery										

References

Abbott NJ, Friedman A (2012) Overview and introduction: the blood-brain barrier in health and disease. Epilepsia 53 Suppl 6: 1-6.

Abraira VE, Ginty DD (2013) The sensory neurons of touch. Neuron 79: 618-39.

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413: 732-8.

Arriola V, Rozelle J (2016) Traumatic Brain Injury in United States Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF) Hispanic Veterans—A Review Using the PRISMA Method. Behavioral Sciences 6: 3.

Arrode-Bruses G, Bruses JL (2012) Maternal immune activation by poly I:C induces expression of cytokines IL-1beta and IL-13, chemokine MCP-1 and colony stimulating factor VEGF in fetal mouse brain. Journal of neuroinflammation 9: 83.

Bailey KR, Crawley JN (2009) Frontiers in Neuroscience
Anxiety-Related Behaviors in Mice. In: Buccafusco JJ (ed) Methods of Behavior Analysis in Neuroscience. CRC Press/Taylor & Francis
Taylor & Francis Group, LLC., Boca Raton (FL)

Bakos J, Duncko R, Makatsori A, Pirnik Z, Kiss A, Jezova D (2004) Prenatal immune challenge affects growth, behavior, and brain dopamine in offspring. AnnNYAcadSci 1018: 281-287.

Banta Lavenex P, Lavenex P (2009) Spatial memory and the monkey hippocampus: not all space is created equal. Hippocampus 19: 8-19.

Beloosesky R, Maravi N, Weiner Z, Khatib N, Awad N, Boles J, Ross MG, Itskovitz-Eldor J (2010) Maternal lipopolysaccharide-induced inflammation during pregnancy programs impaired offspring innate immune responses. AmJ ObstetGynecol 203: 185-4.

Bichler O, Zhao W, Alibart F, Pleutin S, Lenfant S, Vuillaume D, Gamrat C (2013) Pavlov's dog associative learning demonstrated on synaptic-like organic transistors. Neural computation 25: 549-66.

Bird CM, Burgess N (2008) The hippocampus and memory: insights from spatial processing. Nat Rev Neurosci 9: 182-94.

Boksa P (2010) Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain BehavImmun 24: 881-897.

Bonetto A, Andersson DC, Waning DL (2015) Assessment of muscle mass and strength in mice. Bonekey Rep 4: 732.

Boyd BJ, Galle A, Daglas M, Rosenfeld JV, Medcalf R (2015) Traumatic brain injury opens blood-brain barrier to stealth liposomes via an enhanced permeability and retention (EPR)-like effect. Journal of drug targeting 23: 847-53.

Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156: 234-258.

Broadbent NJ, Gaskin S, Squire LR, Clark RE (2010) Object recognition memory and the rodent hippocampus. Learn Mem 17: 5-11.

Bronson SL, Ahlbrand R, Horn PS, Kern JR, Richtand NM (2011) Individual differences in maternal response to immune challenge predict offspring behavior: Contribution of environmental factors. Behav Brain Res 220: 55-64.

Brown AS, Patterson PH (2011) Maternal infection and schizophrenia: implications for prevention. SchizophrBull 37: 284-290.

Bryant R (2011) Post-traumatic stress disorder vs traumatic brain injury. Dialogues in clinical neuroscience 13: 251-62.

Budinich CS, Tucker LB, Lowe D, Rosenberger JG, McCabe JT (2013) Short and long-term motor and behavioral effects of diazoxide and dimethyl sulfoxide administration in the mouse after traumatic brain injury. Pharmacology, biochemistry, and behavior 108: 66-73.

Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35: 625-41.

Cadenhead KS (2011) Startle reactivity and prepulse inhibition in prodromal and early psychosis: effects of age, antipsychotics, tobacco and cannabis in a vulnerable population. Psychiatry Res 188: 208-16.

Carlson CG, Rutter J, Bledsoe C, Singh R, Hoff H, Bruemmer K, Sesti J, Gatti F, Berge J, McCarthy L (2010) A simple protocol for assessing inter-trial and inter-examiner reliability for two noninvasive measures of limb muscle strength. J Neurosci Methods 186: 226-30.

Carroll LJ, Cassidy JD, Holm L, Kraus J, Coronado VG (2004) Methodological issues and research recommendations for mild traumatic brain injury: the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. Journal of rehabilitation medicine: 113-25.

Carter RJ, Morton J, Dunnett SB (2001) Motor coordination and balance in rodents. Curr Protoc Neurosci Chapter 8: Unit 8.12.

Cheney PD (1985) Role of cerebral cortex in voluntary movements. A review. Physical therapy 65: 624-35.

Chodobski A, Zink BJ, Szmydynger-Chodobska J (2011) Blood-brain barrier pathophysiology in traumatic brain injury. Transl Stroke Res 2: 492-516.

Corps KN, Roth TL, McGavern DB (2015) Inflammation and neuroprotection in traumatic brain injury. JAMA neurology 72: 355-62.

Corrigan JD, Whiteneck G, Mellick D (2004) Perceived needs following traumatic brain injury. The Journal of head trauma rehabilitation 19: 205-16.

Crawley JN (1985) Exploratory behavior models of anxiety in mice. Neuroscience and biobehavioral reviews 9: 37-44.

Crawley JN (2007) The Next Generation, in What's Wrong With My Mouse?: Behavioral Phenotyping of Transgenic and Knockout Mice, Second edn. John Wiley & Sons, Inc., Hoboken, New Jersey

Curzon P, Zhang M, Radek RJ, Fox GB (2009) Frontiers in Neuroscience
The Behavioral Assessment of Sensorimotor Processes in the Mouse: Acoustic Startle, Sensory Gating, Locomotor Activity, Rotarod, and Beam Walking. In: Buccafusco JJ (ed) Methods of Behavior Analysis in Neuroscience. CRC Press/Taylor & Francis
Taylor & Francis Group, LLC., Boca Raton (FL)

Dalla C, Shors TJ (2009) Sex differences in learning processes of classical and operant conditioning. Physiology & behavior 97: 229-38.

Das M, Mohapatra S, Mohapatra SS (2012) New perspectives on central and peripheral immune responses to acute traumatic brain injury. Journal of neuroinflammation 9: 236.

DeWitt I, Rauschecker JP (2013) Wernicke's Area Revisited: Parallel Streams and Word Processing. Brain and language 127: 181-91.

Doble A (1999) The role of excitotoxicity in neurodegenerative disease: implications for therapy. Pharmacology & therapeutics 81: 163-221.

Dong XX, Wang Y, Qin ZH (2009) Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta Pharmacol Sin 30: 379-87.

Doran SJ, Ritzel RM, Glaser EP, Henry RJ, Faden AI, Loane DJ (2019) Sex Differences in Acute Neuroinflammation after Experimental Traumatic Brain Injury Are Mediated by Infiltrating Myeloid Cells. Journal of neurotrauma 36: 1040-1053.

Dronkers NF, Plaisant O, Iba-Zizen MT, Cabanis EA (2007) Paul Broca's historic cases: high resolution MR imaging of the brains of Leborgne and Lelong. Brain 130: 1432-41.

Elliott TR, Hsiao YY, Kimbrel NA, Meyer EC, DeBeer BB, Gulliver SB, Kwok OM, Morissette SB (2015) Resilience, traumatic brain injury, depression, and posttraumatic stress among Iraq/Afghanistan war veterans. Rehabilitation psychology 60: 263-76.

Eve DJ, Steele MR, Sanberg PR, Borlongan CV (2016) Hyperbaric oxygen therapy as a potential treatment for post-traumatic stress disorder associated with traumatic brain injury. Neuropsychiatric disease and treatment 12: 2689-2705.

Fluiter K, Opperhuizen AL, Morgan BP, Baas F, Ramaglia V (2014) Inhibition of the membrane attack complex of the complement system reduces secondary neuroaxonal loss and promotes neurologic recovery after traumatic brain injury in mice. Journal of immunology (Baltimore, Md : 1950) 192: 2339-48.

Fortier ME, Kent S, Ashdown H, Poole S, Boksa P, Luheshi GN (2004) The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. AmJ Physiol RegulIntegrComp Physiol 287: R759-R766.

Fortier ME, Luheshi GN, Boksa P (2007) Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. Behav Brain Res 181: 270-277.

Freedman R, Adler LE, Waldo MC, Pachtman E, Franks RD (1983) Neurophysiological evidence for a defect in inhibitory pathways in schizophrenia: comparison of medicated and drug-free patients. Biol Psychiatry 18: 537-51.

Garay PA, Hsiao EY, Patterson PH, McAllister AK (2013) Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. Brain, behavior, and immunity 31: 54-68.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156: 117-154.

Globus MY, Alonso O, Dietrich WD, Busto R, Ginsberg MD (1995) Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. Journal of neurochemistry 65: 1704-11.

Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 12: 238-248.

Gyoneva S, Ransohoff RM (2015) Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell-cell communication by chemokines. Trends Pharmacol Sci 36: 471-80.

Hagenbuch N, Feldon J, Yee BK (2006) Use of the elevated plus-maze test with opaque or transparent walls in the detection of mouse strain differences and the anxiolytic effects of diazepam. Behavioural pharmacology 17: 31-41.

Hall ED, Bryant YD, Cho W, Sullivan PG (2008) Evolution of post-traumatic neurodegeneration after controlled cortical impact traumatic brain injury in mice and rats as assessed by the de Olmos silver and fluorojade staining methods. Journal of neurotrauma 25: 235-47.

Harrison FE, Hosseini AH, McDonald MP (2009) Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. Behav Brain Res 198: 247-51.

Hasenkamp W, Epstein MP, Green A, Wilcox L, Boshoven W, Lewison B, Duncan E (2010) Heritability of acoustic startle magnitude, prepulse inhibition, and startle latency in schizophrenia and control families. Psychiatry Res 178: 236-43.

Heldt SA, Elberger AJ, Deng Y, Guley NH, Del Mar N, Rogers J, Choi GW, Ferrell J, Rex TS, Honig MG, Reiner A (2014) A novel closed-head model of mild traumatic brain injury caused by primary overpressure blast to the cranium produces sustained emotional deficits in mice. Front Neurol 5: 2.

Hergenroeder GW, Moore AN, McCoy JP, Jr., Samsel L, Ward NH, 3rd, Clifton GL, Dash PK (2010) Serum IL-6: a candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury. Journal of neuroinflammation 7: 19.

Hiebert JB, Shen Q, Thimmesch AR, Pierce JD (2015) Traumatic brain injury and mitochondrial dysfunction. The American journal of the medical sciences 350: 132-8.

Hill CA, Threlkeld SW, Fitch RH (2011) Early testosterone modulated sex differences in behavioral outcome following neonatal hypoxia ischemia in rats. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 29: 381-8.

Hodyl NA, Krivanek KM, Clifton VL, Hodgson DM (2008) Innate immune dysfunction in the neonatal rat following prenatal endotoxin exposure. JNeuroimmunol 204: 126-130.

Hodyl NA, Krivanek KM, Lawrence E, Clifton VL, Hodgson DM (2007) Prenatal exposure to a pro-inflammatory stimulus causes delays in the development of the innate immune response to LPS in the offspring. JNeuroimmunol 190: 61-71.

Hsiao EY, McBride SW, Chow J, Mazmanian SK, Patterson PH (2012) Modeling an autism risk factor in mice leads to permanent immune dysregulation. Proceedings of the National Academy of Sciences of the United States of America 109: 12776-81.

Hsiao EY, Patterson PH (2011) Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. Brain, behavior, and immunity 25: 604-15.

Hsiao EY, Patterson PH (2012) Placental regulation of maternal-fetal interactions and brain development. Dev Neurobiol 72: 1317-26.

Hsueh PT, Lin HH, Wang HH, Liu CL, Ni WF, Liu JK, Chang HH, Sun DS, Chen YS, Chen YL (2018) Immune imbalance of global gene expression, and cytokine, chemokine and selectin levels in the brains of offspring with social deficits via maternal immune activation. Genes, brain, and behavior 17: e12479.

Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC (2007) The impact of traumatic brain injuries: a global perspective. NeuroRehabilitation 22: 341-53.

Hynd MR, Scott HL, Dodd PR (2004) Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochemistry international 45: 583-95.

Isaac L, Main KL, Soman S, Gotlib IH, Furst AJ, Kinoshita LM, Fairchild JK, Yesavage JA, Ashford JW, Bayley PJ, Adamson MM (2015) The impact of depression on Veterans with PTSD and traumatic brain injury: a diffusion tensor imaging study. Biological psychology 105: 20-8.

Ison JR, Allen PD (2007) Pre- but not post-menopausal female CBA/CaJ mice show less prepulse inhibition than male mice of the same age. Behav Brain Res 185: 76-81.

Johnston AL, File SE (1991) Sex differences in animal tests of anxiety. Physiology & behavior 49: 245-50.

Jones NC, Cardamone L, Williams JP, Salzberg MR, Myers D, O'Brien TJ (2008) Experimental traumatic brain injury induces a pervasive hyperanxious phenotype in rats. Journal of neurotrauma 25: 1367-74.

Jovanovic T, Szilagyi S, Chakravorty S, Fiallos AM, Lewison BJ, Parwani A, Schwartz MP, Gonzenbach S, Rotrosen JP, Duncan EJ (2004) Menstrual cycle phase effects on prepulse inhibition of acoustic startle. Psychophysiology 41: 401-6.

Jungner M, Siemund R, Venturoli D, Reinstrup P, W SC, Bentzer P (2016) Blood-brain barrier permeability following traumatic brain injury. Minerva anestesiologica 82: 525-33.

Kinkead B, Yan F, Owens MJ, Nemeroff CB (2008) Endogenous neurotensin is involved in estrous cycle related alterations in prepulse inhibition of the acoustic startle reflex in female rats. Psychoneuroendocrinology 33: 178-87.

Klein J, Hadar R, Gotz T, Manner A, Eberhardt C, Baldassarri J, Schmidt TT, Kupsch A, Heinz A, Morgenstern R, Schneider M, Weiner I, Winter C (2013) Mapping brain regions in which deep brain stimulation affects schizophrenia-like behavior in two rat models of schizophrenia. Brain stimulation 6: 490-9.

Koch M (1998) Sensorimotor gating changes across the estrous cycle in female rats. Physiology & behavior 64: 625-8.

Koenig JI, Elmer GI, Shepard PD, Lee PR, Mayo C, Joy B, Hercher E, Brady DL (2005) Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behav Brain Res 156: 251-61.

Koizumi H, Fujisawa H, Ito H, Maekawa T, Di X, Bullock R (1997) Effects of mild hypothermia on cerebral blood flow-independent changes in cortical extracellular levels of amino acids following contusion trauma in the rat. Brain Res 747: 304-12.

Kumar A, Zhang J, Yu FS (2006) Toll-like receptor 3 agonist poly(I:C)-induced antiviral response in human corneal epithelial cells. Immunology 117: 11-21.

Kumari V, Aasen I, Sharma T (2004) Sex differences in prepulse inhibition deficits in chronic schizophrenia. Schizophrenia research 69: 219-235.

Lalonde R, Strazielle C (2003) Motor coordination, exploration, and spatial learning in a natural mouse mutation (nervous) with Purkinje cell degeneration. Behavior genetics 33: 59-66.

Lavenex PB, Amaral DG, Lavenex P (2006) Hippocampal lesion prevents spatial relational learning in adult macaque monkeys. J Neurosci 26: 4546-58.

Lee TS, Mumford D, Romero R, Lamme VA (1998) The role of the primary visual cortex in higher level vision. Vision Res 38: 2429-54.

Levchakov A, Linder-Ganz E, Raghupathi R, Margulies SS, Gefen A (2006) Computational studies of strain exposures in neonate and mature rat brains during closed head impact. Journal of neurotrauma 23: 1570-80.

Lifshitz J, Sullivan PG, Hovda DA, Wieloch T, McIntosh TK (2004) Mitochondrial damage and dysfunction in traumatic brain injury. Mitochondrion 4: 705-13.

Lins BR, Hurtubise JL, Roebuck AJ, Marks WN, Zabder NK, Scott GA, Greba Q, Dawicki W, Zhang X, Rudulier CD, Gordon JR, Howland JG (2018) Prospective Analysis of the Effects of Maternal Immune Activation on Rat Cytokines during Pregnancy and Behavior of the Male Offspring Relevant to Schizophrenia. eNeuro 5.

Lins BR, Marks WN, Zabder NK, Greba Q, Howland JG (2019) Maternal Immune Activation during Pregnancy Alters the Behavior Profile of Female Offspring of Sprague Dawley Rats. eNeuro 6.

Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, Borlongan CV (2015) Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatric disease and treatment 11: 97-106.

Luong TN, Carlisle HJ, Southwell A, Patterson PH (2011) Assessment of motor balance and coordination in mice using the balance beam. Journal of visualized experiments : JoVE.

Malkesman O, Tucker LB, Ozl J, McCabe JT (2013) Traumatic brain injury - modeling neuropsychiatric symptoms in rodents. Front Neurol 4: 157.

Mao H, Jin X, Zhang L, Yang KH, Igarashi T, Noble-Haeusslein LJ, King AI (2010a) Finite element analysis of controlled cortical impact-induced cell loss. Journal of neurotrauma 27: 877-88.

Mao H, Yang KH, King AI, Yang K (2010b) Computational neurotrauma--design, simulation, and analysis of controlled cortical impact model. Biomechanics and modeling in mechanobiology 9: 763-72.

Mattei D, Djodari-Irani A, Hadar R, Pelz A, de Cossio LF, Goetz T, Matyash M, Kettenmann H, Winter C, Wolf SA (2014) Minocycline rescues decrease in neurogenesis, increase in microglia cytokines and deficits in sensorimotor gating in an animal model of schizophrenia. Brain, behavior, and immunity 38: 175-84.

McAllister TW (2008) Neurobehavioral sequelae of traumatic brain injury: evaluation and management. World psychiatry : official journal of the World Psychiatric Association 7: 3-10.

Meehan C, Harms L, Frost JD, Barreto R, Todd J, Schall U, Shannon Weickert C, Zavitsanou K, Michie PT, Hodgson DM (2017) Effects of immune activation during early or late gestation on schizophrenia-related behaviour in adult rat offspring. Brain, behavior, and immunity 63: 8-20.

Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. Eur J Pharmacol 698: 6-18.

Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. The Journal of nutrition 130: 1007s-15s.

Meyer U, Feldon J, Schedlowski M, Yee BK (2005) Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. Neurosci BiobehavRev 29: 913-947.

Meyer U, Feldon J, Schedlowski M, Yee BK (2006a) Immunological stress at the maternal-foetal interface: a link between neurodevelopment and adult psychopathology. Brain BehavImmun 20: 378-388.

Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, Yee BK, Feldon J (2006b) The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J Neurosci 26: 4752-4762.

Minambres E, Cemborain A, Sanchez-Velasco P, Gandarillas M, Diaz-Reganon G, Sanchez-Gonzalez U, Leyva-Cobian F (2003) Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. Crit Care Med 31: 933-8.

Missault S, Van den Eynde K, Vanden Berghe W, Fransen E, Weeren A, Timmermans JP, Kumar-Singh S, Dedeurwaerdere S (2014) The risk for behavioural deficits is determined by the maternal immune response to prenatal immune challenge in a neurodevelopmental model. Brain, behavior, and immunity 42: 138-46.

Moreno JL, Kurita M, Holloway T, Lopez J, Cadagan R, Martinez-Sobrido L, Garcia-Sastre A, Gonzalez-Maeso J (2011) Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT(2)A and mGlu(2) receptors in the adult offspring. J Neurosci 31: 1863-72.

Morissette SB, Woodward M, Kimbrel NA, Meyer EC, Kruse MI, Dolan S, Gulliver SB (2011) Deployment-related TBI, persistent postconcussive symptoms, PTSD, and depression in OEF/OIF veterans. Rehabilitation psychology 56: 340-50.

Morton SM, Bastian AJ (2004) Cerebellar control of balance and locomotion. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 10: 247-59.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learn Mem 9: 49-57.

Mutinati M, Pantaleo M, Roncetti M, Piccinno M, Rizzo A, Sciorsci RL (2014) Oxidative stress in neonatology: a review. Reproduction in domestic animals = Zuchthygiene 49: 7-16.

Mychasiuk R, Hehar H, Farran A, Esser MJ (2014) Mean girls: sex differences in the effects of mild traumatic brain injury on the social dynamics of juvenile rat play behaviour. Behav Brain Res 259: 284-91.

Niesman IR, Schilling JM, Shapiro LA, Kellerhals SE, Bonds JA, Kleschevnikov AM, Cui W, Voong A, Krajewski S, Ali SS, Roth DM, Patel HH, Patel PM, Head BP (2014) Traumatic brain injury enhances neuroinflammation and lesion volume in caveolin deficient mice. Journal of neuroinflammation 11: 39.

Nilsson P, Hillered L, Ponten U, Ungerstedt U (1990) Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. J Cereb Blood Flow Metab 10: 631-7.

Niyonkuru C, Wagner AK, Ozawa H, Amin K, Goyal A, Fabio A (2013) Group-based trajectory analysis applications for prognostic biomarker model development in severe TBI: a practical example. Journal of neurotrauma 30: 938-45.

Obrenovitch TP, Urenjak J (1997) Is high extracellular glutamate the key to excitotoxicity in traumatic brain injury? Journal of neurotrauma 14: 677-98.

Ong WY, Tanaka K, Dawe GS, Ittner LM, Farooqui AA (2013) Slow excitotoxicity in Alzheimer's disease. Journal of Alzheimer's disease : JAD 35: 643-68.

Onore CE, Schwartzer JJ, Careaga M, Berman RF, Ashwood P (2014) Maternal immune activation leads to activated inflammatory macrophages in offspring. Brain, behavior, and immunity 38: 220-6.

Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M (2006) Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. BiolPsychiatry 59: 546-554.

Pagulayan KF, Temkin NR, Machamer J, Dikmen SS (2006) A longitudinal study of health-related quality of life after traumatic brain injury. Archives of physical medicine and rehabilitation 87: 611-8.

Patrich E, Piontkewitz Y, Peretz A, Weiner I, Attali B (2016) Maternal immune activation produces neonatal excitability defects in offspring hippocampal neurons from pregnant rats treated with poly I:C. Sci Rep 6: 19106.

Patterson PH (2002) Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. CurrOpinNeurobiol 12: 115-118.

Patterson PH (2009) Immune involvement in schizophrenia and autism: etiology, pathology and animal models. BehavBrain Res 204: 313-321.

Patterson PH, Xu W, Smith SE, Devarman BE (2008) Maternal immune activation, cytokines, and autism. In: Zimmerman AW (ed) Autism. Humana Press, Totowa, New Jersey, pp 289-307

Pavlov PI (2010) Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Annals of neurosciences 17: 136-41.

Paxinos G, Franklin KBJ (2004) The Mouse Brain in Stereotaxic Coordinates, Second edn. Academic Press, San Diego

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14: 149-67.

Plappert CF, Rodenbucher AM, Pilz PK (2005) Effects of sex and estrous cycle on modulation of the acoustic startle response in mice. Physiology & behavior 84: 585-94.

Pritchard LM, Logue AD, Hayes S, Welge JA, Xu M, Zhang J, Berger SP, Richtand NM (2003) 7-OH-DPAT and PD 128907 Selectively Activate the D3 Dopamine Receptor in a Novel Environment. Neuropsychopharmacology 28: 100-107.

Quillinan N, Herson PS, Traystman RJ (2016) Neuropathophysiology of Brain Injury. Anesthesiology clinics 34: 453-64.

Rahman T, Zavitsanou K, Purves-Tyson T, Harms LR, Meehan C, Schall U, Todd J, Hodgson DM, Michie PT, Weickert CS (2017) Effects of Immune Activation during Early or Late Gestation on N-Methyl-d-Aspartate Receptor Measures in Adult Rat Offspring. Frontiers in psychiatry 8: 77.

Rao VL, Dogan A, Todd KG, Bowen KK, Dempsey RJ (2001) Neuroprotection by memantine, a non-competitive NMDA receptor antagonist after traumatic brain injury in rats. Brain Res 911: 96-100.

Reger ML, Poulos AM, Buen F, Giza CC, Hovda DA, Fanselow MS (2012) Concussive brain injury enhances fear learning and excitatory processes in the amygdala. Biol Psychiatry 71: 335-43.

Reisinger S, Khan D, Kong E, Berger A, Pollak A, Pollak DD (2015) The Poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. Pharmacology & therapeutics.

Richtand NM, Ahlbrand R, Horn P, Stanford K, Bronson SL, McNamara RK (2011) Effects of risperidone and paliperidone pre-treatment on locomotor response following prenatal immune activation. JPsychiatrRes 45: 1194-1201.

Richtand NM, Ahlbrand R, Horn P, Tambyraja R, Grainger M, Bronson SL, McNamara RK (2012a) Fluoxetine and Aripiprazole Treatment Following Prenatal Immune Activation Exert Longstanding Effects on Rat Locomotor Response. PhysiolBehav 106: 171-177.

Richtand NM, Ahlbrand R, Horn PS, Chambers B, Davis J, Benoit S (2012b) Effects of prenatal immune activation and peri-adolescent stress on amphetamine-induced conditioned place preference in the rat. Psychopharmacology (Berl) 222: 313-24.

Risbrough V, Ji B, Hauger R, Zhou X (2014) Generation and characterization of humanized mice carrying COMT158 Met/Val alleles. Neuropsychopharmacology 39: 1823-32.

Risdall JE, Menon DK (2011) Traumatic brain injury. Philosophical transactions of the Royal Society of London Series B, Biological sciences 366: 241-50.

Romine J, Gao X, Chen J (2014) Controlled cortical impact model for traumatic brain injury. Journal of visualized experiments : JoVE: e51781.

Ruff RM, Iverson GL, Barth JT, Bush SS, Broshek DK (2009) Recommendations for diagnosing a mild traumatic brain injury: a National Academy of Neuropsychology education paper. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists 24: 3-10.

Russell KL, Kutchko KM, Fowler SC, Berman NE, Levant B (2011) Sensorimotor behavioral tests for use in a juvenile rat model of traumatic brain injury: assessment of sex differences. J Neurosci Methods 199: 214-22.

Salinska E, Danysz W, Lazarewicz JW (2005) The role of excitotoxicity in neurodegeneration. Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences 43: 322-39.

Sangha S, Greba Q, Robinson PD, Ballendine SA, Howland JG (2014) Heightened fear in response to a safety cue and extinguished fear cue in a rat model of maternal immune activation. Frontiers in behavioral neuroscience 8: 168.

Schwarzbold ML, Rial D, De Bem T, Machado DG, Cunha MP, dos Santos AA, dos Santos DB, Figueiredo CP, Farina M, Goldfeder EM, Rodrigues AL, Prediger RD, Walz R (2010) Effects of traumatic brain injury of different severities on emotional, cognitive, and oxidative stress-related parameters in mice. Journal of neurotrauma 27: 1883-93.

Schwendener S, Meyer U, Feldon J (2009) Deficient maternal care resulting from immunological stress during pregnancy is associated with a sex-dependent enhancement of conditioned fear in the offspring. Journal of neurodevelopmental disorders 1: 15-32.

Scott SH (2003) The role of primary motor cortex in goal-directed movements: insights from neurophysiological studies on non-human primates. Curr Opin Neurobiol 13: 671-7.

Shi L, Fatemi SH, Sidwell RW, Patterson PH (2003) Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 23: 297-302.

Shin Yim Y, Park A, Berrios J, Lafourcade M, Pascual LM, Soares N, Yeon Kim J, Kim S, Kim H, Waisman A, Littman DR, Wickersham IR, Harnett MT, Huh JR, Choi GB (2017) Reversing behavioural abnormalities in mice exposed to maternal inflammation. Nature 549: 482-487.

Shoji H, Takao K, Hattori S, Miyakawa T (2016) Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. Mol Brain 9: 11.

Siopi E, Llufriu-Daben G, Fanucchi F, Plotkine M, Marchand-Leroux C, Jafarian-Tehrani M (2012) Evaluation of late cognitive impairment and anxiety states following traumatic brain injury in mice: the effect of minocycline. Neurosci Lett 511: 110-5.

Smith SE, Li J, Garbett K, Mirnics K, Patterson PH (2007) Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 27: 10695-10702.

Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS (2005) The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. Journal of psychopharmacology 19: 221-7.

Sternbach GL (2000) The Glasgow coma scale. The Journal of emergency medicine 19: 67-71.

Swerdlow NR, Caine SB, Braff DL, Geyer MA (1992) The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. Journal of psychopharmacology 6: 176-90.

Swerdlow NR, Hartman PL, Auerbach PP (1997) Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. Biol Psychiatry 41: 452-60.

Taylor BC, Hagel EM, Carlson KF, Cifu DX, Cutting A, Bidelspach DE, Sayer NA (2012) Prevalence and costs of co-occurring traumatic brain injury with and without psychiatric disturbance and pain among Afghanistan and Iraq War Veteran V.A. users. Medical care 50: 342-6.

Teasdale G, Maas A, Lecky F, Manley G, Stocchetti N, Murray G (2014) The Glasgow Coma Scale at 40 years: standing the test of time. The Lancet Neurology 13: 844-54.

Utagawa A, Truettner JS, Dietrich WD, Bramlett HM (2008) Systemic inflammation exacerbates behavioral and histopathological consequences of isolated traumatic brain injury in rats. Exp Neurol 211: 283-91.

Van den Eynde K, Missault S, Fransen E, Raeymaekers L, Willems R, Drinkenburg W, Timmermans JP, Kumar-Singh S, Dedeurwaerdere S (2014) Hypolocomotive behaviour associated with increased microglia in a prenatal immune activation model with relevance to schizophrenia. Behav Brain Res 258: 179-86.

Van den Hove DL, Lauder JM, Scheepens A, Prickaerts J, Blanco CE, Steinbusch HW (2006) Prenatal stress in the rat alters 5-HT1A receptor binding in the ventral hippocampus. Brain Res 1090: 29-34.

Vorhees CV, Graham DL, Braun AA, Schaefer TL, Skelton MR, Richtand NM, Williams MT (2012) Prenatal immune challenge in rats: altered responses to dopaminergic and glutamatergic agents, prepulse inhibition of acoustic startle, and reduced route-based learning as a function of maternal body weight gain after prenatal exposure to poly IC. Synapse 66: 725-37.

Vorhees CV, Graham DL, Braun AA, Schaefer TL, Skelton MR, Richtand NM, Williams MT (2015) Prenatal immune challenge in rats: Effects of polyinosinic-polycytidylic acid on spatial learning, prepulse inhibition, conditioned fear, and responses to MK-801 and amphetamine. Neurotoxicology and teratology 47: 54-65.

Wagner AK, Kline AE, Ren D, Willard LA, Wenger MK, Zafonte RD, Dixon CE (2007) Gender associations with chronic methylphenidate treatment and behavioral performance following experimental traumatic brain injury. Behav Brain Res 181: 200-9.

Wang H, Zhang YP, Cai J, Shields LB, Tuchek CA, Shi R, Li J, Shields CB, Xu XM (2016) A Compact Blast-Induced Traumatic Brain Injury Model in Mice. Journal of neuropathology and experimental neurology 75: 183-96.

Wang KC, Fan LW, Kaizaki A, Pang Y, Cai Z, Tien LT (2013) Neonatal lipopolysaccharide exposure induces long-lasting learning impairment, less anxiety-like response and hippocampal injury in adult rats. Neuroscience 234: 146-57.

Warden D (2006) Military TBI during the Iraq and Afghanistan wars. The Journal of head trauma rehabilitation 21: 398-402.

Warrier C, Wong P, Penhune V, Zatorre R, Parrish T, Abrams D, Kraus N (2009) Relating structure to function: Heschl's gyrus and acoustic processing. J Neurosci 29: 61-9.

Washington PM, Forcelli PA, Wilkins T, Zapple DN, Parsadanian M, Burns MP (2012) The effect of injury severity on behavior: a phenotypic study of cognitive and emotional deficits after mild, moderate, and severe controlled cortical impact injury in mice. Journal of neurotrauma 29: 2283-96.

Weinstock M (2007) Gender differences in the effects of prenatal stress on brain development and behaviour. Neurochemical research 32: 1730-40.

Witcher KG, Eiferman DS, Godbout JP (2015) Priming the inflammatory pump of the CNS after traumatic brain injury. Trends Neurosci 38: 609-620.

Wolff AR, Bilkey DK (2010) The maternal immune activation (MIA) model of schizophrenia produces pre-pulse inhibition (PPI) deficits in both juvenile and adult rats but these effects are not associated with maternal weight loss. Behav Brain Res 213: 323-327.

Woodcock T, Morganti-Kossmann MC (2013) The role of markers of inflammation in traumatic brain injury. Front Neurol 4: 18.

Xuan IC, Hampson DR (2014) Gender-dependent effects of maternal immune activation on the behavior of mouse offspring. PloS one 9: e104433.

Yang SH, Gangidine M, Pritts TA, Goodman MD, Lentsch AB (2013a) Interleukin 6 mediates neuroinflammation and motor coordination deficits after mild traumatic brain injury and brief hypoxia in mice. Shock (Augusta, Ga) 40: 471-5.

Yang SH, Gustafson J, Gangidine M, Stepien D, Schuster R, Pritts TA, Goodman MD, Remick DG, Lentsch AB (2013b) A murine model of mild traumatic brain injury exhibiting cognitive and motor deficits. The Journal of surgical research 184: 981-8.

Yu S, Kaneko Y, Bae E, Stahl CE, Wang Y, van Loveren H, Sanberg PR, Borlongan CV (2009) Severity of controlled cortical impact traumatic brain injury in rats and mice dictates degree of behavioral deficits. Brain Res 1287: 157-63.

Yurgil KA, Barkauskas DA, Vasterling JJ, Nievergelt CM, Larson GE, Schork NJ, Litz BT, Nash WP, Baker DG, Marine Resiliency Study T (2014) Association between traumatic brain injury and risk of posttraumatic stress disorder in active-duty Marines. JAMA psychiatry 71: 149-57.

Zuckerman L, Rehavi M, Nachman R, Weiner I (2003) Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. Neuropsychopharmacology 28: 1778-1789.

Zuckerman L, Weiner I (2005) Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. J Psychiatr Res 39: 311-323.