Development of Rat Concussion Model of TBI and Comparative Behavioural Effects to Blast-induced traumatic brain injury (biTBI)

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Prepared for Dr. Yushan Wang, Technical Authority

For CiMVHR contract Task 30, 2013

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Yushan Wang, MD, PhD
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DRDC Suffield

March 17, 2014

Re: Progess Report for CIMVHR/ICRSMV Task 30

Dear Dr. Wang:

Includeed please find a progress report for the study entitled "**Development of Rat Concussion Model of TBI and Comparative Behavioural Effects to Blast-induced traumatic brain injury (biTBI) (Project Task 30).**

The report includes a breakdown in the methodology to induce the concussion in animals of a similar age and strain to those that your group will be using to study the effects of blast injury. Also included is some data outlioning the behavioural effects of our concussion model. The tissue has been harvested for maolecular analysis but due to time constraints has not yet been processed. Once I have this data, I will forward it to you and your group.

As you will be able to quickly tell from the data there are some very interesting findings that we hope will be of use for comparison to the effects of blast injury. In short, our platform has been validatedand is now ready to be used for comparative studies directed at your model and population. We look forward to further collaboration with you and your group to explore the mechnistic underpinnings of these models in an effort to improve the outcomes of military personel and civilians after TBI

Sincerely,

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Project Summary.

All experiments were carried out in accordance with the Canadian Council of Animal Care and were approved by the University of Calgary Conjoint Faculties Research Ethics Approval Board. Sprague Dawley rats were ordered from Charles Rivers Laboratories (Charles River Laboratories, Wilmington, MA, USA) and litters used for this study were derived from in-house breeding pairs (*primiparous*). Upon arrival, animals were housed in groups of 3 or 4 and maintained on a 12:12-h light:dark cycle in a quiet temperature controlled (21˚C) husbandry room. Animals had access to food and water *ad libitum*, and were handled daily by an experimenter in order to reduce the stress associated with transportation and familiarize the animals to the experimenter.

On P60, animals received a single mTBI (Kane et al.,2012; Mychasiuk et al., 2014a and b) or a sham injury (Male mTBI n = 4, Male Sham n = 44, Female mTBI n = 4, Female Sham n = 4). Briefly, the mTBI was induced using a modified weight drop technique. Animals were anesthetized lightly until there was an absence of peripheral nociceptive response, i.e. lack of toe pinch response, and quickly placed chest down upon a stage consisting of a slit piece of aluminum foil 10 cm above a sponge cushion. A weight (150 grams) (tethered to the instrument with commercially available knotless Nylon 8 lb angler fishing line) was dropped from 0.5 meters through a PVC guide tube (20 mm diameter x 1.5 m length) and permitted to impact the head of the anesthetized rat. Upon impact, the rat underwent a complete 180˚ horizontal rotation and free fell onto the sponge cushion. The weight was tethered to the instrument with fishing line to ensure that the weight did not travel further than 1cm beyond the dorsal surface of the head and could not produce "re-hits" to the head or body of the rat. Immediately after impact, topical lidocaine was applied to the head of the rat and they were placed in a clean cage set upon a warm heating pad to recover. Animals experiencing a sham injury were lightly anesthetized, placed chest down on the aluminum foil stage, but were removed without the impacting weight or the rotational free-fall. They also received application of topical lidocaine to the head and were placed in clean cages on warm heating pads to recover. The time each rat took to arouse from anesthetic and elicit and appropriate righting reflex was recorded. Time to right was measured as the time from weight impact/sham impact until the rat had rotated from a supine position to a prone/standing position. Once the rats were behaving in a typical manner (walking, exploring, etc.) they were returned to their home cages.

Behavioural Testing

For 15 days following the mTBI, all animals were subjected to a comprehensive behavioural test battery to examine balance and motor coordination, cognitive functioning, spatial learning, and emotionality. The behavioural test battery included time to right, beam walking, open field (locomotor activity), Novel context mismatch (NCM), elevated plus maze (EPM), and the forced swim task (FS).

Beam Walking. Twenty-four hours post-mTBI (P61) animals were tested in a beam walking task similar to that described by Shallert et al. (2002). The rat's home-cage was placed at one end of the 165cm long tapered beam and the start point was the other wider end of the beam. The tapered beam had ledges (2 cm wide) that provide "safety" when the rat's foot slipped off of the beam. The rat was given a single trial to learn to walk from the start of the beam to its home-cage. Once the rat reached its home-cage, it was permitted to remain in the cage for 60 seconds to reinforce the target location. The rat was given four additional videotaped trials, each separated by the 60s reinforcement. The video camera was set to record from the starting point of the beam and positioned to view down the length of the beam. A researcher blinded to the experimental groups, scored the videos for hind leg foot slips off the tapered beam onto the safety ledges.

Elevated plus maze (EPM). One day post-mTBI (P61) animals were also tested in the EPM. The EPM was constructed from black Plexiglas® and consisted of two open arms and two closed arms that are elevated 55 cm above the ground. The maze was located in an empty room and video recording occurred with the lights on. The video camera was placed at the end of one of the open arms in a slightly elevated position. Rats were placed in the centre of the platform with their front paws facing a closed arm. Rats were videotaped for 10 minutes and

scored by a research analyst blinded to the experimental conditions for the amount of time rats spent in the closed and open arms of the platform, as well as time spent in the centre of the maze. The EPM was cleaned with Virkon after each rat completed the task.

Open field. Two days post-mTBI (P62) animals were tested in the open field task. Rats were individually placed into a 100 x 100 x 50 cm square and allowed to explore their environment for 10 minutes. An overhead-tracking camera was used to track the rat's movement and measure distance travelled as well as time spent in the outer portion of the square and the inner portion of the square. The open field box was cleaned with Virkon after each rat completed the task.

Novel Context Mismatch (NCM). From P65-38 animals were tested in the NCM task similar to that described by Spanswick and Sutherland (2010). Rats were exposed to two different contexts for 5 min each day, one immediately after the other, for 3 consecutive days. Context A was a clear plastic rectangular box (70 x 40 x 33 cm) and context B was a dark circular bin (diameter 47 x 36 cm high). For the three days of context learning, each context housed a unique pair of identical objects (context A – 2 Rubik's cubes, context B – 2 50 ml conical tubes), securely attached to the floor of the context, that the rats explored for 5 minutes. On the probe day, rats were permitted to explore each of the normal contexts, one after another for five minutes. After a 5 minute delay, the rats were videotaped while they were exposed to a modified context (Context A with 1 object from context A and 1 from context B; or Context B with 1 object from context B and 1 object from context A) for 5 minutes. A research analyst blinded to the experimental conditions scored the amount of time the rat spent with the old object and the amount of time spent with the new/novel object.

Forced swim task (FS). On P75 animals were tested in a modified forced swim task similar to that described by Yadid et al. (2001). A cylindrical tank (30 cm diameter x 60 cm deep) was filled with warm water (~25˚C) to a level that the rat's tail could not touch the bottom of the tank. The rat was placed in the tank and videotaped for 7 minutes. Upon completion of the task the rat was dried with a warm towel and returned to his home-cage. The tank was emptied and re-filled with clean warm (~25˚C) water between each test session. A research analyst blinded to the experimental conditions scored the videos for the amount of time each rat spent immobile (not swimming, just floating).

Molecular analysis.

Following completion of all behavioural testing, animals were terminally anesthetised with sodium pentobarbital, decapitated and their brain flash frozen in liquid nitrogen. The brains were then placed in storage at -80C for later analysis of patterns of molecular changes in gene expression using either quantitative pcr of western immunoblot analysis. This analysis is on-going but because of the timing of the proposal we were not able to complete it. Results will be forwarded as they are competed and analysed.

Statistical Analysis.

As described above, all scoring of behavioural measures were completed by a research analyst blinded to the experimental conditions. Two-way ANOVAs with Sex (male and female) and Injury (mTBI and sham) as factors were run for each of the behavioural outcomes measured and *p* values less than .05 were considered statistically significant. SPSS 19.0 for Mac was used to complete all analyses.

Results.

Behavioural Testing

Time to Right (Figure 1). A measurement of the time it takes an animal to right immediately following the mTBI vs waking from the anesthetic alone. The two-way ANOVA revealed a main effect of injury, $F(1, 11) = 8.30$, $p = .02$, but not of sex, $F(1, 11) = 0.03$, $p = .87$. The interaction was not significant, $F(1, 11) = 0.22$, $p = .65$.

Beam Walk Footslips (Figure 2). This test examines motor coordination 24 hours following the mTBI using a beam walking task that measures the number of hind leg footslips. Although the two-way ANOVA did not reveal a significant main effect, the trend is strong and indicates that a greater sample size may reveal a significant effect. The two-way ANOVA demonstrated a trend toward an injury effect, $F(1, 11) = 3.88$, $p = .08$, no effect of sex, $F(1, 11) = 0.05$, $p = .83$, and no significant interaction, $F(1, 11) = 0.89$, $p = .37$.

Distance Covered in the Openfield (Figure 3). The open field is primarily a measure of locomotor activity. This testing session measured the distance each rat travelled in an open field environment over a 10 minute period. The two-way ANOVA demonstrated that animals with a mTBI are less active and travel significantly less distance, $F(1, 11) = 39.88$, $p < .01$. In addition, similar to an abundance of literature, there is a significant sex effect that demonstrates that females are more active than males, $F(1, 11) = 14.44$, $p < .01$. The interaction was not significant, $F(1, 11) = 0.67$, $p = .44$.

*Novel Context Mismatch (Figure 4).*The novel context mismatch paradigm is a short-term working memory task that involves the prefrontal cortex and the hippocampus. Measurements were recorded for the amount of time that animals spent exploring the two objects in the test session and the amount of time the animals spent with the novel object. The two-way ANOVA for exploration time demonstrated that male rats with a mTBI at P60 spent significantly more time than any of the other rats exploring the objects, (sex by injury interaction, $F(1, 11) = 6.39$, $p = .03$). Despite this increase in exploration time, rats that had experienced a mTBI at P60 could not differentiate the novel object from the familiar object. The two-way ANOVA for time with the novel object demonstrated a main effect of injury, $F(1, 11) = 37.27$, $p < .01$, but not of sex, $F(1, 11) = 3.06$, $p = .12$. The interaction was also not significant, $F(1, 11) = 3.67$, $p = .09$.

Elevated Plus Maze (Figure 5). The elevated plus maze was carried out 72 hours post mTBI and is generally used to measure anxiety in rodents. There is a negative correlation between time in the open arms and anxiety, whereby as time in the open arms decreases, anxiety is believed to increase. The two-way ANOVA for time spent in the open arms demonstrated a main effect of injury, $F(1, 11) = 9.96$, $p = .01$, and a main effect of sex, $F(1, 11) = 7.27$, $p = .03$. The interaction was not significant, $F(1, 11) = 2.88$, $p = .13$. See Figure 5.

Forced Swim Paradigm (Figure 6). The forced swim paradigm is used as a measure for depression; the more animals are depressed the greater amount of time they spend immobile in the testing session. The two-way ANOVA for the percentage of time each rat spent immobile demonstrated no significant main effects of injury $F(1, 11) = 0.29$, $p = .64$, or sex, $F(1, 11) = 0.18$, $p = .68$ and the interaction was not significant, $F(1, 11) = 0.59$, $p = .46$. See Figure 6.

Summary.

As demonstrated in the Results, we have been able to successfully adapt our modelling platform to induce a mTBI in older animals (roughly equivalent to human 17-19 year olds). The meaningful behavioral outcomes are significant as compared to controls and suggest impairment in motor function, cognition, mood and affect following TBI. This impairment is persistent as demonstrated by abnormalities 2 weeks after injury suggestive of features of post-concussive syndrome (allowing for age extrapolation between species). The results also suggest a sex-specific magnitude of effect; something that we have seen in younger animals (Mychasiuk et al., 2014a)

Further studies will; (1) expand on the number of animals in each group to increase the robustness of the data, (2) analyze the harvested brains for changes in gene or protein expression related to hypothesis of underlying pathogenic mechanisms (i.e. aberrations in the cellular stress response), and compare and contrast outcomes to those induced by your model of primary blast injury.

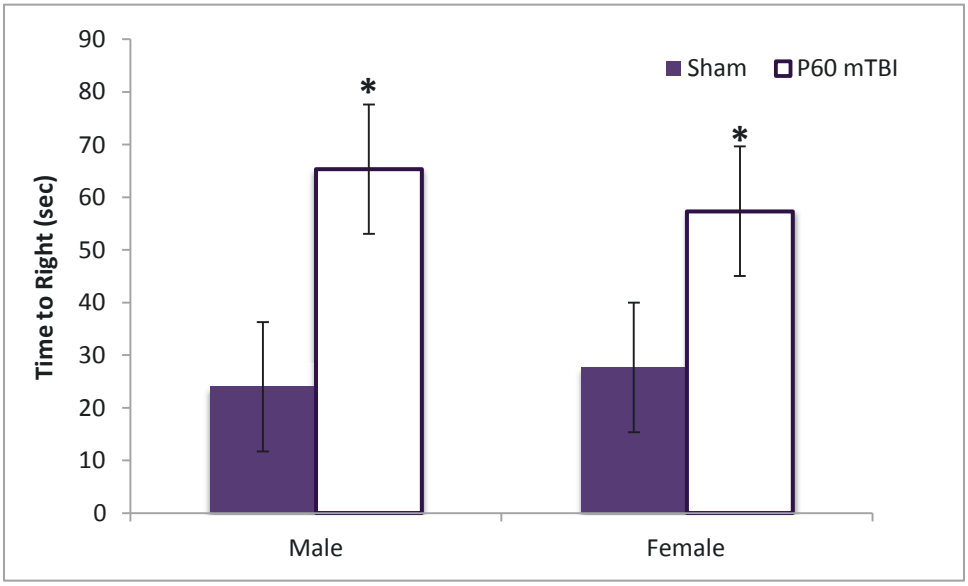

Figure 1. An illustrative demonstration of the average ***time to right*** (seconds) between animals with an mTBI at P60 or a sham injury (*p =.02).

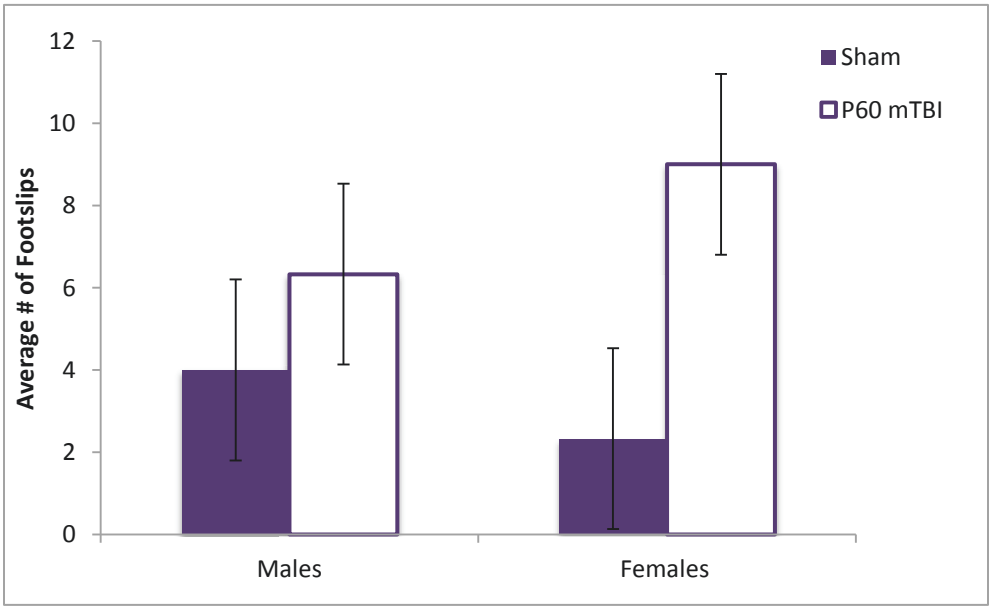

Figure 2. Illustrative representation of the average number of ***hind-leg footslips*** in animals 24 hours post mTBI.

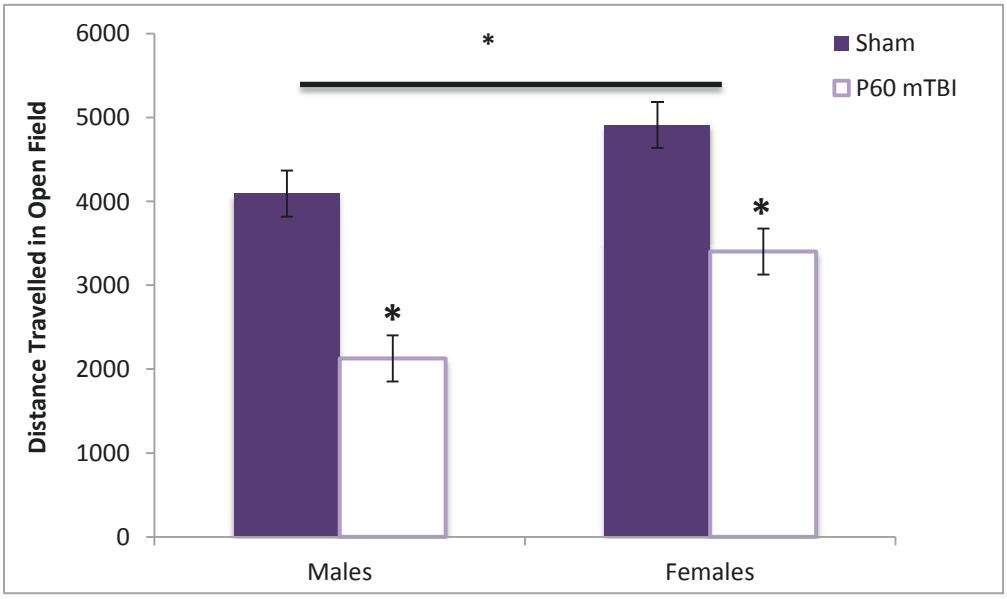

Figure 3. Illustrative representation of the distance rats traveled in the ***openfield*** 48 hours post-mTBI demonstrating that injured rats were less active than control rats.

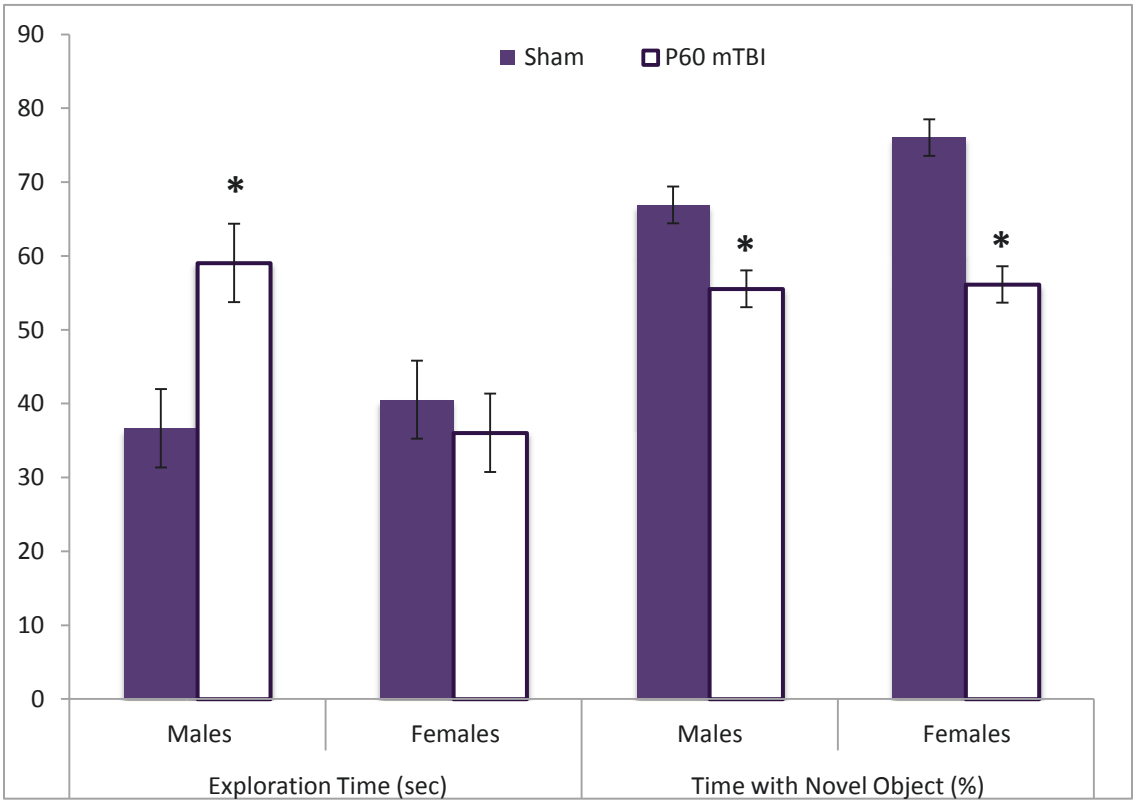

Figure 4. Illustration of the results from the ***novel context mismatch*** task that demonstrate that rats with a mTBI at P60 cannot differentiate between the novel object and the familiar object. Interestingly, when compared to all rats, male rats with a mTBI spend significantly more time actually exploring the objects (*$p < .05$).

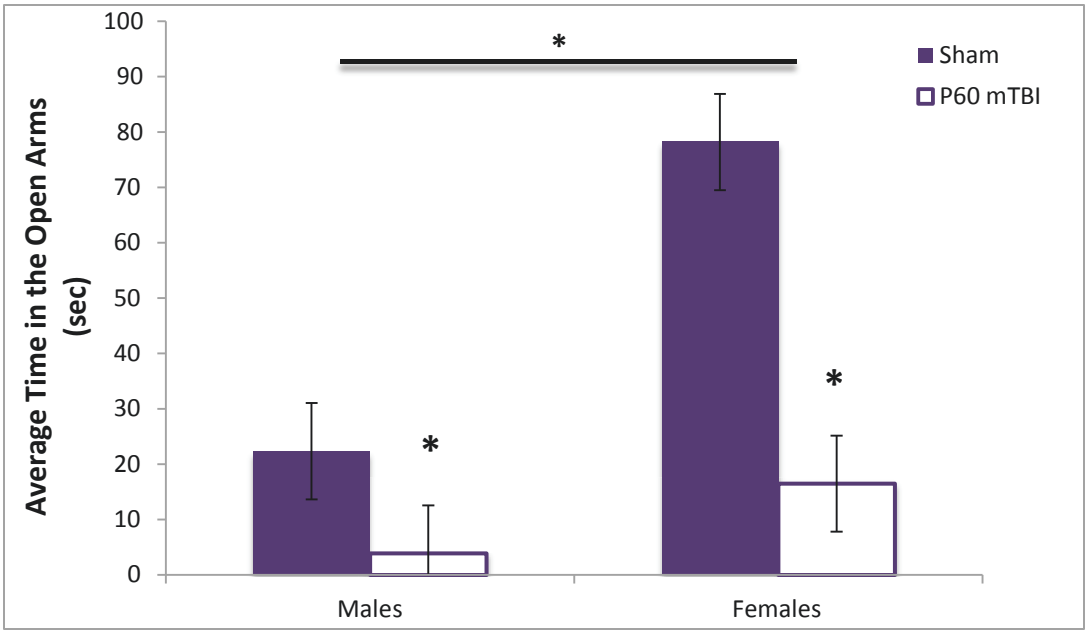

Figure 5. Graphical representation of the time male and female rats with a mTBI at P60 or a sham injury spend in the open arms of the ***elevated plus maze*** (*$p < .05$).

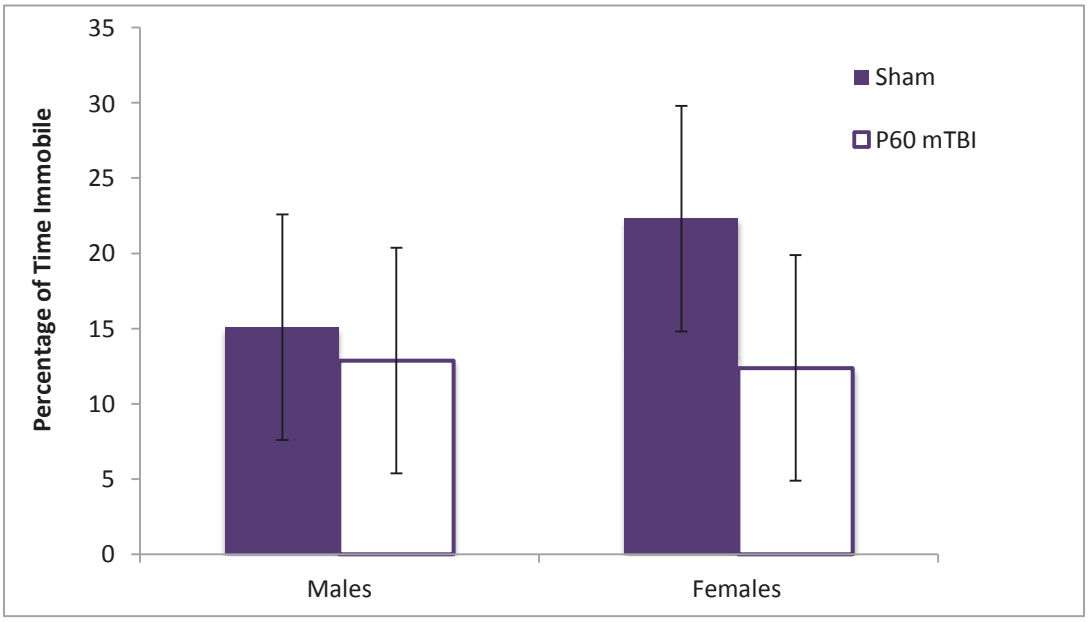

Figure 6. Graphical representation of the time male and female rats spent immobile in the ***forced swim paradigm***.

Select References.

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