The Effect of Peripheral Nerve-Injury on Depression and Anxiety-Like Behaviours in Mice

by

Erinn Leah Acland

A thesis submitted in conformity with the requirements for the degree of Master of Arts

Psychology Department
University of Toronto

© Copyright by Erinn L. Acland 2017
The Effect of Peripheral Nerve-Injury on Depression and Anxiety-Like Behaviours in Mice

Erinn Leah Acland

Master of Arts
Psychology Department
University of Toronto

2017

Abstract

Past animal studies examining the relationship between depression and chronic pain have used only male rodents and often only assessed behaviours 7 to 14 days after an injury. To determine whether chronic pain results in a sexually dimorphic presentation of depression-like behaviours, I conducted a series of experiments assessing male and female mice at 14, 28, and 42 days after a peripheral nerve injury. I found that mice did not show any changes in behaviours 14 or 28 days post-injury. At 42 days post-surgery, male mice with a nerve injury showed significantly more depressive-like behaviours than the sham group, however females did not. This suggests that results from male rodents may not be generalizable to females and that studies assessing mental health and chronic pain in rodents should assess behaviour over longer periods of time as to be more representative of long-term pain experiences.
Acknowledgments

The author thanks Dr. Loren Martin for his support and guidance with this project and Meruba Sivaselvachandran for helping with the behavioural experiments. I would also like to thank the UTCSP for awarding me with the Pain Scientist Scholarship to support my continued research on this subject. Funds were provided by the SGS Conference Grant, the UTM Conference Travel Grant, and the UTM Psychology Graduate Student Travel Grant to present this research at the Canadian Pain Society conference in 2016. Lastly, I would like to thank Peter Duggan for building much of the equipment necessary for these experiments.
Table of Contents

Acknowledgments .......................................................................................................... iii

Table of Contents ........................................................................................................ iv

List of Figures ............................................................................................................. vi

Chapter 1: INTRODUCTION......................................................................................... 1

1 Background ................................................................................................................ 1

1.1 Shared physiology ................................................................................................. 1

1.2 Rodent models of comorbid pain and depression ................................................. 2

1.3 Sex differences ....................................................................................................... 2

Chapter 2: OBJECTIVES AND HYPOTHESES ....................................................... 5

Chapter 3: METHODOLOGY ...................................................................................... 6

3 Experimental Design ................................................................................................. 6

3.1 Animal Care ........................................................................................................... 6

3.2 Surgery Protocol ................................................................................................... 6

3.3 Behavioural Tests .................................................................................................. 7

3.3.1 Von Frey filament test ...................................................................................... 7

3.3.2 Open field test ................................................................................................ 7

3.3.3 Tail suspension test ......................................................................................... 8

3.4 Data Analysis ........................................................................................................ 9

Chapter 4: RESULTS .................................................................................................. 10

4 Outcomes .................................................................................................................. 10

4.1 Baseline Characteristics ....................................................................................... 10

4.1.1 Outliers ........................................................................................................... 10

4.1.2 Sex Differences .............................................................................................. 11

4.2 Timepoint Condition ........................................................................................... 12

4.2.1 Sex Differences .............................................................................................. 13

iv
4.3 Experimenter Effect ...........................................................................................................15

Chapter 5: DISCUSSION ........................................................................................................17

5 Conclusions .........................................................................................................................17

5.1 Replication of literature .................................................................................................17

5.2 Sex differences ...............................................................................................................18

5.3 Tester Effects ..................................................................................................................18

5.4 Summary ..........................................................................................................................19

References ..............................................................................................................................20
List of Figures

Figure 1. The open field test ........................................................................................................ 8
Figure 2. The tail suspension test ................................................................................................. 8
Figure 3. Analysis of outlier ......................................................................................................... 10
Figure 4. Baseline sex differences in behaviours ......................................................................... 11
Figure 5. Baseline sex differences in distance moved ................................................................. 11
Figure 6. Changes in behaviour at 14 and 28 days post-surgery ................................................. 12
Figure 7. Changes in behaviour at 42 days post-surgery ............................................................ 13
Figure 8. Sex differences in changes in behaviour at 14, 28, and 42 days post-surgery .......... 14
Figure 9. Correlation between hindpaw sensitivity and immobility at 42 days post-surgery .... 14
Figure 10. Effect of tester on open field test .............................................................................. 15
Figure 11. Effect of tester on tail suspension test ....................................................................... 16
Figure 12. Effect of tester on baseline behaviours .................................................................... 16
Chapter 1

INTRODUCTION

Pain is essential for avoiding bodily harm, however chronic pain is a maladaptive state where pain occurs spontaneously and sensations are amplified. More than 50% of people with chronic pain also suffer from depression and are twice as likely to commit suicide (Fishbain, Goldberg, Meagher, Steele, & Rosomoff, 1986; Fishbain, Goldberg, Rosomoff, & Rosomoff, 1991). The development of chronic pain and depression are intimately linked and the combination of them leads to higher health care costs, lower quality of life, and worse treatment outcomes (Arnow et al., 2006; Burns, Kubilus, Bruehl, Harden, & Lofland, 2003).

1 Background

1.1 Shared physiology

Nerve damage and dysfunction induces a series of physiological changes starting at the site of injury leading all the way up to the brain. In the cerebrum after nerve injury, it has been found that nociceptive input into the amygdala increases, the anterior cingulate has long-term synaptic changes, and the prefrontal cortex and brain stem have upregulation of proinflammatory cytokine gene expression (Apkarian et al., 2006; Neugebauer, Li, Bird, & Han, 2004; Xu et al., 2008). These changes are thought to facilitate nociceptive signals through activation of efferent pain pathways, which consist of the midbrain periaqueductal grey (PAG) and rostroventral medulla (Norman et al., 2010). Activation of inflammatory pathways in the brain have been associated with decreased neurotrophic support, altered glutamate release/reuptake, and oxidative stress, all of which are characteristic of those with depressive disorders (Miller, Maletic, & Raison, 2009). Additionally, those diagnosed with major depression have increased expression of peripheral blood inflammatory cytokines (Miller et al., 2009). Proinflammatory cytokines, such as interleukin-1β (IL-1β), have been shown to induce both hyperalgesia (hypersensitivity to noxious stimuli) and depression-like behaviour in animals (Watkins & Maier, 2005). Additionally, administration of the proinflammatory cytokine interferon-α results in depression in up to 50% of clinical patients (Raison, Capuron, & Miller, 2006). These studies would indicate that inflammatory cytokines play a key role in the co-development of depression and neuropathic
pain. Therefore, the favoured theory for the high comorbidity of pain and depression is that neuroinflammation induced by chronic pain facilitates the development of depression or vice versa.

1.2 Rodent models of comorbid pain and depression

Rodent models have been utilized to study the relationship between depression and neuropathic pain. For example, Norman et al. (2010) found that mice showed increased depressive behaviours 7 days after a nerve injury. They also showed that these mice had increased IL-1β gene expression in the frontal cortex and when an IL-1 receptor antagonist was administered, depressive behaviours decreased. This experiment supports the neuroinflammation pain-depression theory, however two other studies showed that at 14 days post-nerve injury, rodents showed no significant increase in depressive behaviours when compared to sham surgery animals (Kontinen, Kauppila, Paananen, Pertovaara, & Kalso, 1999; Urban, Goulding, Tecott, & Basbaum, 2011). Urban et al., (2011) showed that even at around 42 days post-surgery sham and nerve-injury groups did not significantly differ in depressive behaviours in the forced swim or sucrose preference test. All these experiments used the forced swim test, which measures immobility as an indication of depressive behaviours. Therefore, it is possible that mice at 7 days post-surgery move less due to their continued recovery from surgery and by 14 days the rodents have improved mobility, thereby increasing struggling behaviour. One other crucial issue with all these rodent experiments is that they only used male rodents, even though the majority of those suffering from chronic pain in human populations are females.

1.3 Sex differences

Despite the high prevalence of pain disorders in the general populous, the neurobiology of how chronic pain develops and is maintained is poorly understood and even less is known about how it differs between sexes. Women have been shown to develop chronic pain and comorbid depression/anxiety more frequently than men, however it is unknown what is mediating this discrepancy in prevalence rates (Mogil, 2012; Tsang et al., 2008). This lack of understanding about why sex differences occur in pain populations is partially due to researchers using only male rodents to study pain (Greenspan et al., 2007; Mogil, 2012). It was found that 79% of studies published in the journal PAIN over a 10-year period used only male animals in their
experiments (Mogil & Chanda, 2005). There is a pervasive belief in the research community that female animals have more variability in behaviour due to their estrus cycle’s hormonal fluctuations, which make it more difficult to achieve significant results. However, an analysis of behaviour variability done by Mogil and Chanda (2005) showed that it was actually males that tended to have more variable behaviour. Avoiding using female animals in pain research is not only unfounded but also impedes the discovery of important sexual dimorphisms in pain processes that could have implications on the treatment of chronic pain. Some researchers are championing this issue and replicating seminal papers with females to confirm their validity across sexes. Although, some new studies are suggesting that significant differences exist in the way males and females develop chronic pain. For example, a study by Deleo, Tanga, and Tawfik (2004) showed that microglia were involved in the development of neuropathic pain in rodents. However, a study published over a decade later showed that female rodents use an entirely different immune cell (T-cells) for the development of neuropathic pain, which was not previously discovered simply because the first study only used male rodents (Sorge et al., 2015).

1.3.1 Anxiety-like behaviours in rodents

Human anxiety disorders are usually defined as excessive, exaggerated worry, or nervousness about non-threatening events. Avoidant and hypervigilant behaviours are thought to represent the experience of fear and/or anxiety. Thereby, freezing behaviour, increased defecation, and reduced exploratory behaviour are thought to represent fear and anxiety-related experiences in rodents. Tests that examine anxiety-like behaviours in rodents have consistently shown differences between females and males. Beatty (1979) observed that male rats show baseline increased defecation and less overall activity than females. Additionally, results from elevated plus maze indicate that female rats spend more time in open areas than males indicating that they may have lower baseline anxiety (Johnson & File, 1991). Some studies have shown that there are not only baseline differences in responding to these tests but also sexually dimorphic responses to drug and stress administration. Meng and Drugan (1993), showed that administering the anxiogenic compound FG 7142 (20 mg/kg) significantly decreased male exploratory behaviour, however females need twice the dose (40 mg/kg) as males to show the same effect. Another study showed that chronic exposure to inescapable shocks produced a reduction in number of entries into open areas by male rodents, but not females (Steenbergen, Heinsbroek, Van Hest, &
Van de Poll, 1990). These studies suggest that male and female rodents have differing baselines of anxiety-like behaviour and changes in response to a stimulus can be sex-dependent.

1.3.2 Depression-like behaviours in rodents

Depression in humans is associated with a diverse set of behaviours, such as trouble doing normal day-to-day tasks, reduced pleasure experience (i.e., anhedonia), changes in eating behaviour, changes in sleep, changes in activity, and fatigue among others. A few of the most common tests used for assessing depression in rodents are the forced swim, tail suspension, and sucrose-preference tests. The forced swim and tail suspension tests measure learned helplessness, while the sucrose-preference test assesses anhedonia. Immobility and reduced sugar-water consumption in these tests are thought to indicate depression-like behaviours. Studies assessing baseline sex differences in the forced swim test have shown that female rats show less immobility and higher activity levels than males (Alonso, Castellano, Afonso, & Rodriguez, 1991; Barros & Ferigolo, 1998; Brotto, Barr, & Gorzalka, 2000). These results are maintained regardless of the females’ stage in the estrus cycle (Alonso et al., 1991). Interestingly, the opposite is found in the tail suspension test, where females spend significantly more time immobile than males (Liu & Gershenfeld, 2001). Similar to anxiety behaviours, depressive behaviours can show sexually dimorphic outcomes in response to stimuli. For example, one study showed that when melatonin was administrated; males had increased struggling behaviours in the forced swim test, while females showed no effect of melatonin (Brotto et al., 2000). Another study showed that when mice were isolated, females showed increased immobility in the forced swim test, while immobility in isolated males decreased (Palanza, Gioiosa, & Parmigiani, 2001). This demonstrates again the potential effects sex can have on behaviour, further confirming that anxiety and depressive results from male rodents are not necessarily generalizable to female rodents.
Chapter 2
OBJECTIVES AND HYPOTHESES

The main goal of my Master’s project was to perform a set of experiments that assessed depressive and anxiety-like behaviours in male and female rodents at 14, 28, and 42 days after a peripheral nerve injury. These experiments were performed to determine whether the results found in past experiments that used male rodents could be generalized to females. The objectives of my experiments were to (1) replicate past studies that showed no increases in depressive behaviours in males at 14, 28, and 42 days post-nerve injury (2) replicate findings from experiments that used the forced swim and sucrose preference with the tail suspension test (3) determine whether females display similar levels of depressive and anxious behaviours as male rodents. Since past studies have shown no significant increases in depressive behaviours in males from 14 days post-nerve injury onwards, I predicted that male rodents would show no differences in depressive behaviours between nerve injury and sham groups at all time points. Additionally, I hypothesized that female rodents with a nerve injury would show increased depressive-like behaviours since females tend to exhibit high pain-depression comorbidity in human populations.
Chapter 3

METHODOLOGY

3 Experimental Design

To determine whether the male and female rodents have similar behavioural responses to persistent hypersensitivity, we assessed mice on a battery of behavioural tests, then performed nerve injury or sham surgeries on them. After which, mice were re-assessed using the same pre-surgical behavioural tests at either 14, 28, or 42 days post-surgery (not repeated measures design). Each group had an N=6-9 (e.g., female sham group at 42 days n=6-9). Mice were run in same-sex groups of 8, where half had a nerve injury and half had sham surgeries in order to blind the experimenters.

3.1 Animal care

Naïve CD-1 male and female mice were either ordered from Charles River or bred in-house. Mice ordered from outside sources were habituated to the animal room for at least one week prior to experimentation and were housed in cages with 3 to 5 same-sex siblings. All mice were at least 6 weeks old, females weighed between 20 to 34g and males weighed between 27 to 53g at baseline. Mice were kept in a temperature control room (23+/−1 °C) and were maintained at a 12 hour of light cycle (8am to 8pm).

3.2 Surgery protocol

Spared-nerve injury (SNI) surgeries were performed on the mice to induce persistent hypersensitivity. SNI is a surgery where two out of the three terminal branches of the sciatic nerve are severed causing increased pressure sensitivity in the ipsilateral hindpaw. Mice were anesthetized using an induction chamber with 3% isoflurane and then maintained throughout the surgery with 2 to 2.5% isoflurane. The hindpaw thigh area was shaved and disinfected using ethanol and iodine sequentially. Using a scalpel, an incision approximately 5mm long was made through which, 2 branches of the sciatic nerve were severed below a ligation of the nerve. The skin was sutured and recovery was monitored for two days post-surgery. There was also a group
of sham surgery mice for every experiment, where the skin and muscle was cut identically to the surgery condition such that the nerve was exposed, but was not severed. I performed all sham and SNI surgeries for these experiments.

3.3 Behaviour Tests

The hypersensitivity, depression and anxiety-like behaviours of the mice were assessed through a battery of behavioural tests. The order for performing these tests were: von frey, open field, then tail suspension test. Mice were placed back into their home cages (with water and food available) for 5 to 10 minutes in between tests. The behaviour tests were performed either the day before or the same day as the SNI or sham surgery and then again either 14, 28, or 42 days post-surgery to assess changes.

3.3.1 Von Frey filament test

To confirm hypersensitivity in the affected hindpaw, we performed the von Frey filament test the morning before or the morning of the surgery (baseline measure) and again on the morning of the endpoint along with the other behaviour tests. The von Frey filament test is used to assess mechanical pain thresholds. All mice were habituated in the testing cubicles for 1 to 1.5 hours prior to assessment. The automatic von Frey machine applies a filament to the hindpaw of the mouse and increases the pressure until the mouse withdraws its paw. The number of grams of pressure applied that induced a hindpaw withdrawal was recorded. Each mouse had both hindpaws measured for sensitivity five times (5 to 10 minutes between each measure). The responses were then averaged and used as a measure of hindpaw sensitivity for a particular point in time.

3.3.2 Open field test

The open field test measures anxiety in rodents by assessing the amount of time spent in the center of an empty box versus close to the walls over a period of 6 minutes. Mice were placed in boxes 40 x 40 x 40 cm and were recorded and analyzed by EthoVision, which is a visual analysis software that can measure a variety of behaviours. The boxes were divided into a 4 by 4 grid, the
4 center squares in the grid were considered “open areas”, while the 12 boxes near the walls were analyzed as “wall areas”.

![Figure 1](image1.png)

**Figure 1.** The open field test assesses anxiety-like behaviour by recording the time spent in the center of the box (highlighted red area) over a 6-minute period.

### 3.3.3 Tail suspension test

The tail suspension test is a measure of learned helplessness that monitors the amount of time spent immobile when suspended by the tail over 6 minutes. This test was performed by applying a small piece of tape to the end of the tail and clamping the tape to a hook in a 40 x 40 x 40 cm box. The subsequent behaviour was recorded using a video camera, then analyzed for immobility using EthoVision.

![Figure 2](image2.png)

**Figure 2.** The tail suspension test assesses depressive-like behaviour in rodents by recording the time spent immobile over a 6-minute period of suspension. The tape was attached to a hook (not the ceiling of the box).
3.4 Data Analysis

All analyses used an $\alpha=0.05$ (95% confidence interval). To assess the effects of time point condition on behavioural outcomes, independent two-tailed t-tests were performed for each time point where the independent variable was surgery condition and the dependent variables were change in time spent immobile and in open areas. To examine the effects of tester on time point results, a two-way ANOVA was performed assessing their effect on change in open field and tail suspension tests. Independent t-tests were performed to test whether there were significant baseline differences in tail suspension and open field tests between experimenters EA and MS.
Chapter 4

RESULTS

4 Outcomes

A total of 93 CD-1 mice were used in this experiment, one of which was excluded from analysis due to being an outlier in the tail suspension test. All experiments were performed between January and September 2016.

4.1 Baseline Characteristics

4.1.1 Outliers

To identify any mice with baseline outlier behaviour, we used SPSS’s outlier identifier feature. I assessed all data together to identify outlier behaviour in time spent immobile and time spent in open areas at baseline. One outlier was identified; a male in the 42-day post-surgery endpoint group. The mouse’s baseline immobility was 239.5 seconds, which was 2.6 standard deviations above the mean (M=96.65, SD=54.79, n=92). We excluded the outlier from all analysis.

Figure 3. Analysis of outlier baseline (BL) behaviour in tail suspension and open field tests identified one outlier in the male 42-day group (ID 32), which was excluded from all subsequent analysis.
4.1.2 Sex Differences

An independent samples two-tailed t-test was performed on pre-surgery measures of immobility and exploratory behaviour to assess whether there was baseline sex difference in the tests as reported by previous studies. We found that female mice (M=117.26, SD=54.29) spent significantly more time (s) immobile during baseline tail suspension than males (M=74.51, SD=45.78; t(91) = -4.091, p < 0.000, see Figure 4). No significant sex differences in time spent open areas were found (t(90) = 1.714, p = 0.09). We also found that males moved slightly farther (cm) than females during baseline open field tests, but not significantly (t(90) = 1.929, p = 0.057, see Figure 5).

**Figure 4.** Female mice (n=48) spent significantly more time (s) immobile during baseline tail suspension than males (n=45; t(91) = -4.091, p < 0.000), however no differences were found in time spent in open in the open field test (p > 0.05).

**Figure 5.** Males (n=44) moved slightly farther (cm) than females (n=48) during baseline open field tests, but not significantly (t(90) = 1.929, p = 0.057). Baseline tail suspension test showed no significant differences in distance moved (cm) between sexes (p > 0.05).
4.2 Timepoint Condition

We performed independent two-tailed t-tests for each time point experiment to assess whether surgery condition was associated with changes (from baseline measures) in time spent immobile and time spent in open areas in the tail suspension and open field tests, respectively. We found that at 14 and 28 days post-surgery, mice with a peripheral nerve-injury did not show significant changes in behaviours in the open field test (14 days: $t(29) = -1.272, p = 0.214$; 28 days: $t(27) = -0.203, p = 0.84$) or the tail suspension test (14 days: $t(30) = 0.9, p = 0.375$; 28 days: $t(27) = -0.782, p = 0.441$) when compared with sham groups (see Figure 6). However at 42 days post-surgery, mice with a SNI surgery ($M=42.66, SD=41.56$) spent significantly more time immobile in the tail suspension test than mice with a sham surgery ($M=-8.03, SD=68.47$; $t(29) = -2.471, p = 0.02$ see Figure 7). No significant differences between SNI ($n=15$) and sham ($n=14$) groups were found in the open field test 42 days post-surgery ($t(29) = 0.974, p = 0.338$). These results would suggest that significant depressive-like behaviours develop in SNI mice 42-days post-surgery, when compared to sham surgery mice.

Figure 6. Spared nerve injury (SNI) ($n=17$) and sham ($n=14-15$) surgery mice showed no significant differences between groups at 14 and 28 days post-surgery in change in time spent immobile and in open areas ($p > 0.05$). Behavioural changes were assessed by subtracting post-surgery (PS) results from baseline (BL) measures of each animal, whereby a positive score indicates an increase in that behaviour at the post-surgical assessment point (sec).
Figure 7. Mice showed significant changes ($t(29) = -2.47, p = 0.02$) in time spent immobile in the tail suspension test between SNI ($n=15$) and sham surgery ($n=16$) conditions at 42 days post-surgery, however no significant differences were found in the open field test ($p > 0.05$).

### 4.2.1 Sex Differences

All the results remained the same when analyzed by sex, except for immobility in the 42 days post-surgery groups. When analyzed by sex, male SNI mice ($M=50.92, SD=31.29$) still showed significant changes in immobility when compared to sham mice ($M=-18.53, SD=38.63; t(13) = -3.787, p = 0.002$), while females did not show significant differences ($t(14) = -0.898, p = 0.385$; see Figure 8). However, mice show different degrees of hypersensitivity in response to a nerve injury and if the sural nerve is damaged during the surgery, the mouse’s hindpaw can become numb to sensation instead of being hypersensitive. To determine whether post-surgical hindpaw hypersensitivity was correlated to change in immobility, a bivariate Pearson correlation was performed on each sex within each time point. At 42 days post-surgery both male ($r = -0.549, p = 0.034$) and female ($r = -0.537, p = 0.032$) mice showed significant negative correlations between post-surgical hindpaw sensitivity and change in immobility between baseline and post-surgery tests. No other time points showed any significant correlations ($p > 0.05$). However, post-surgical weight of the mouse was significantly positively correlated to hindpaw sensitivity ($r = 0.301, p = 0.005, n = 85$). Interestingly, when post-surgery weight was controlled for using a partial correlation Pearson test, males’ hindpaw sensitivity remained significantly negatively correlated to change in immobility ($r = -0.575, df = 12, p = 0.031$), however females did not ($r = -0.434, df = 13, p = 0.106$; see Figure 9).
Figure 8. No significant differences between SNI and sham groups (all groups n=6-9) were found at 14 or 28 days post-surgery ($p > 0.05$). At 42 days, SNI male mice (n=7) showed significant ($t(13) = -3.787, p = 0.002$) increases in immobile behaviour in the tail suspension test when compared to sham surgery mice (n=8), however no significant differences were found in SNI vs. sham females ($p > 0.05$). Change in immobility is the post-surgery time spent immobile subtracted from the baseline (BL) time spent immobile.

Figure 9. At 42 days post-surgery, male ($r = -0.575, p = 0.031, n = 15$) but not female ($r = -0.434, p = 0.106, n = 16$) mice showed significant negative correlations between change in immobility behaviour (tail suspension test) and post-surgical hindpaw sensitivity when mouse weight was controlled for.
4.3 Experimenter Effect

Two female experimenters performed the behavioural assays. To assess whether there was an experimenter effect on the results I performed a two-way MANOVA assessing whether there was a main effect of tester or whether tester interacted with time point to effect tail suspension and open field outcomes. I found that there was a significant main effect of tester ($F(1, 55) = 49.78, p < 0.000$) and a significant interaction between tester and time point in open field ($F(2, 55) = 13.44, p < 0.000$, see Figure 10) but not tail suspension tests ($p > 0.05$, see Figure 11). However, when an independent two-tailed t-test was performed on baseline measures of open field ($t(90) = -0.192, p = 0.848$) and tail suspensions ($t(91) = -0.327, p = 0.744$), no significant differences between testers were found (see Figure 12). These findings indicate that the tester-time point interaction is likely due to other factors that varied between groups analyzed.

Figure 10. A significant main effect of tester ($F(1, 55) = 49.78, p < 0.000$) and significant interaction between tester and time point was found to affect change in time spent in open ($F(2, 55) = 13.44, p < 0.000$). Each group tested by both experimenters EA and MS had n=5-8.
Figure 11. No main effect of tester ($F(1, 55) = 2.24, p = 0.14$) or significant interaction between tester and time point was found in change in tail suspension immobility ($F(2, 55) = 2.22, p = 0.119$). Each group tested by both experimenters EA and MS has $n=5-8$.

Figure 12. No significant differences between EA ($n=45$) and MS ($n=48$) testers were found in baseline open field ($t(90) = -0.192, p = 0.848$) and tail suspension tests ($t(91) = -0.327, p = 0.744$).
Chapter 5
DISCUSSION

5 Conclusions

5.1 Replication of literature

Chronic pain conditions have been found to be highly comorbid with depression. Past animal studies assessing the relationship between chronic pain and depression have found conflicting results on whether rodents develop depressive-like behaviours after a nerve injury. My study was aimed at providing further support (one way or the other) on whether rodents are appropriate models for studying the relationship between chronic pain and depression. Previous research has shown that male rodents exhibit depressive-like behaviours 7 days post-SNI in the forced swim test, but not between 14 and 43 days post-SNI in the forced swim or sucrose preference test (Norman et al., 2010; Urban et al., 2011). Using the tail suspension test, I replicated past results that showed that male mice do not exhibit increased depressive-like behaviours at 14 or 28 days post-nerve injury when compared to sham groups. Depression that is comorbid with chronic pain in human populations often does not spontaneously recover, and is defined as lasting a minimum of 2 weeks. Norman et al., (2010) showed that male rodents had increased immobility at 7 days post-nerve injury, however did not assess any later time points. To increase confidence in their findings that inflammatory cytokines mediate depressive behaviours after pain development, their results need to be replicated at later time points and in females. In addition to this, my experiments showed that 42 days post-SNI, male mice were significantly more immobile than sham groups. This conflicts with Urban et al., (2011)’s study which found no increase in depression-like behaviours when compared to sham groups at 40-43 days post-SNI. There are some differences between the studies’ designs, which could have mediated these conflicting results. The most salient differences included the use of different mouse strains (Urban et al., 2011 used Balb/c and C57B1/6), different tests for measuring depressive behaviours and different study designs (Urban et al., 2011 used same mice for each time point). To determine why our results differed we will need to replicate our findings with the forced swim and sucrose preference tests, replicate our findings using repeated measures design, and include several other mouse strains.
5.2 Sex differences

Women are disproportionately affected by both chronic pain and depression. Despite this, animal models studying the interaction between persistent pain and mental health have exclusively used male rodents. I expected my results would mirror female human populations so that female mice would develop more significant depression-like behaviours than males. Surprisingly, my findings showed that female rodents did not show any significant increases in anxious or depressive behaviours 14, 28, or 42 days post-SNI, when compared to sham groups. However, at 42 days post-surgery SNI female mice showed non-significant increases in immobility when compared to sham mice. This may suggest the beginning of development of depressive-like behaviours in females. To confirm this, longer time points post-surgery would need to be performed. My findings suggest that when studying chronic pain and depression, male rodents may not be generalizable to females due to their divergent development of depressive-like behaviours after a nerve injury.

Previous studies have reported that females have increased activity levels, reduced immobility in the forced swim test, and increased immobility in the tail suspension test when compared to male rodents. My results replicated past findings, showing that females displayed significantly more immobility in the tail suspension test at baseline when compared to males. At baseline, males did not differ from females in exploratory behaviours and moved farther distances than female mice in the open field test during baseline measures, although not significantly ($p = 0.057$).

5.3 Tester Effects

Our experiments were analyzed using EthoVision software and experimenters were blind to the surgery conditions minimizing the possibility of tester effects. However, we did find a significant interaction between the two experimenters EA and MS and the time point condition in the open field test. Each experimenter tested groups of cagemate siblings, therefore differences could be due to cage effects or genetic differences. In addition to this, all mice tested by EA were from Charles Rivers laboratories, while when MS started behavioural testing, the lab had switched to breeding mice in-house. This resulted in MS testing overall younger mice (all were minimum 6 weeks old) that did not have the stress of being shipped and were born in a different
environment, which may have contributed to a tester effect. To further confirm that the effect found was likely due to extraneous variables, averaged baselines measures of immobility and exploratory behaviours of all mice between experimenters were assessed and found to be non-significantly different. Furthermore, when results were analyzed with either EA or MS’s findings removed from analysis, results remained the same. Further experiments will need to be conducted to determine what resulted in this significant interaction effect.

5.4 Summary

The relationship between chronic pain and depression in human populations is a developing research area. Studies using animal models to explore this comorbidity have yielded conflicting findings. My research would suggest that depressive-like behaviours do not develop in male mice until at least 42 days post-nerve injury, and female rodents did not exhibit any significant depressive behaviours. This would suggest that studies studying the relationship between chronic pain and depression in male rodents should not assume that their findings generalize to females. In addition to this, my research suggests that studies should use time points 42 days post-surgery or longer to better replicate the development of human depression in those with chronic pain.
References


