SOCIAL STRESS SENSITIZES THEILER'S VIRUS-INDUCED CYTOKINE EXPRESSION

A Thesis

by

MALLORY ANN FRAZIER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2010

Major Subject: Psychology

SOCIAL STRESS SENSITIZES THEILER'S VIRUS-INDUCED CYTOKINE EXPRESSION

A Thesis

by

MALLORY ANN FRAZIER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Mary Meagher
Committee Members,	C. Jane Welsh
	Michelle Hook
Head of Department,	Les Morey

August 2010

Major Subject: Psychology

ABSTRACT

Social Stress Sensitizes Theiler's Virus-induced Cytokine Expresssion.

(August 2010)

Mallory Ann Frazier, B.S, University of Mary Washington

Chair of Advisory Committee: Dr. Mary Meagher

Our laboratory has previously shown that exposure to social disruption (SDR) the week prior to Theiler's murine encephalomyelitis virus (TMEV) infection exacerbates disease course, resulting in increased infection-related sickness behaviors, motor impairment, CNS viral titers, and CNS inflammation. These adverse effects of SDR were prevented by ICV infusion of a neutralizing antibody to IL-6 during the stress exposure period. These findings suggest that stress-induced increases in IL-6 are necessary to exacerbate acute TMEV infection, but the exact mechanism remains unknown. This thesis tested the hypotheses that SDR up-regulates central cytokine expression, exacerbates TMEV infection through cross-sensitization of virus-induced cytokine expression, and that social rank modulates the effect of SDR.

In Experiment 1, Balb/cJ mice underwent the 0, 1, or 6 SDR sessions and were then sacrificed 0, 2, or 12 hours post SDR. Experiment 2 subjects received ICV infusions of either IL-6 neutralizing antibody or its vehicle before each of six 2 h SDR sessions or the control condition, the week prior to infection.

In Experiment 3 mice were tested for pre-existing social rank prior to SDR and infection. Results indicate that (1) SDR increases virus-induced IL-6, IL-1β, and CD11b mRNA expression in brain,that these SDR-induced increases and acute TMEV exacerbation are prevented by ICV infusion of the IL-6 neutralizing antibody during the stress exposure period, and that (2) social rank does not modulate affects of SDR but baseline anxiety does. These findings suggest that SDR exacerbates acute TMEV infection through cross-sensitization of virus-induced cytokine expression and that baseline anxiety is a significant modulator of SDR.

DEDICATION

To my Mom, Dad, and Steve for their support, patience, and love

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Mary Meagher, and my committee members, Dr. Jane Welsh and Dr. Michelle Hook, for their guidance and support throughout the course of this research. This research was supported by NIH/NINDS R01-NS060822.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. I would like to thank all members of the Meagher laboratory, whose hard work and support made this thesis possible. Specific thanks go to Elisabeth Vichaya and Erin Young for their patience, teaching, endless edits, and friendship. I also want to extend my gratitude to Dr Aaron Taylor for statistical guidance and advice.

Finally, thanks to my mom, dad, future husband, and a myriad of friends for their encouragement, patience, and love.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The complex interaction between stress and the immune system has been the focus of much research. In recent years, research in our laboratory has shown that prior exposure to social disruption stress (SDR) exacerbates a mouse model of multiple sclerosis, Theiler's Murine Encephalomyelitis Virus (TMEV) infection, and that the pro-inflamatory cytokine interleukin-6 (IL-6) is necessary for that exacerbation (Johnson et al., 2004, 2006; Meagher et al., 2007). This thesis extended this line of research by testing the hypothesis that prior exposure to stress sensitizes the inflamatory response to TMEV. Additionally, we sought to test the hypothesis that up-regulation of IL-6 is a possible mediator of stress-induced sensitization. Furthermore, given that research in both humans and animals has shown that the psychological and physiological reactions to stress can be highly individualized, we explored potential individual difference variables that may mediate or modulate the effects of stress on infection (Bartolomucci, et al., 2005; Maes et al., 1998). Despite the genetically identical nature of laboratory mice, in our model we have observed that the impact of SDR on disease severity varies considerably between mice. Therefore, in addition to furthering our understanding of stress and immune interactions, this thesis examined whether individual differences in social rank explain the differential effects of SDR on TMEV infection. This thesis was designed to test the hypothesis that repeated exposure to SDR exacerbates acute TMEV

This thesis follows the style of *Behavioral Neuroscience.*

infection through cross-sensitization of pro-inflamatory cytokine expression. It was predicted that both social stress and TMEV infection would activate pro-inflamatory cytokine expression and that prior SDR-induced increases in IL-6 would amplify the central pro-inflamatory cytokine responses to TMEV infection. Further, we hypothesized that pre-established social rank would act as a modulating variable to determine the level of disease exacerbation induced by SDR. The following sections provide background information upon which these hypotheses are built. First, the independent variables are introduced followed by a discussion of the dependent variables and rationale for testing these hypotheses.

Stress, Immune, and Nervous System Interactions

Immune challenges, such as tissue damage, infectious agents, or administration of lipopolysaccharide (LPS; a gram negative endotoxin), much like stress, trigger a complex cascade of events characterized by activation of the sympathetic nervous system and release of the glucocorticoids via the Hypothalamic Pituitary Adrenal (HPA) axis (Elenkov et al., 2005; Engeland et al., 2001; Maier & Watkins, 1998). Furthermore, stress, much like immune challenges, can activate pro-inflamatory cytokine expression. This would suggest that the stress and immune system shares common neural circuitry (Dantzer, 2001; Kelley et al., 2003; Maier & Watkins, 1998). For example, previous research has shown that acute stress results in an inflamatory response similar to that seen with a peripheral immune challenge (Deak et al., 1997; Johnson et al., 2002; O'Conner et al., 2003). Specifically, inescapable tail shock induces systemic and central increases in mRNA expression of pro-inflamatory cytokines, IL-6, interleukin 1-β (IL-

1β), and Tumor Necrosis Factor α (TNFα; O'Connor et al., 2003). These pro-inflamatory cytokines are capable of inducing debilitating sickness behaviors in both human and animal models of stress and immune challenges (Bluthè et al., 2000; Dantzer et al., 1999; Goshen et al., 2008; Kansman, Parnet, & Dantzer, 2001; Kelley et al., 2003; Merali et al., 2003; Mohr et al., 2004; Pollmacher et al., 2001).

Repeated activation of the common neural circuitry underlying reactions to stress and immune challenges results in an enhancement of the inflamatory response and increased pro-inflamatory cytokine release (Frank et al., 2007; Johnson et al., 2002; Maier, 2003; Meagher et al., 2007; Perry, Cunningham, & Holmes , 2007). Recent research indicates that prior exposure to a stressor causes microglia activation (Nair & Bonneau, 2006, Frank et al., 2007) and sensitization of pro-inflamatory cytokines such that subsequent immune challenges then show an enhanced inflamatory response (Johnson et al, 2002; Meagher et al., 2007; Steelman et al., 2009; Quan et al. 2001; Young et al., 2010). Given that macrophages and microglia are the major source of central pro-inflamatory cytokines, it has been suggested that 'priming' of macrophages and microglia is the cellular source for the sensitization of pro-inflamatory cytokine release (Frank et al., 2007; Perry, Cunningham, and Holms, 2007; Perry, Newman, and Cunningham, 2003). Blandino, Barnum, and Deak (2006) provide further evidence of this, by demonstrating that microglial inhibition reverses the shock-induced increases in hypothalamic IL-1β.

Unfortunately, much of the research studying stress-induced CNS cytokine expression has focused on IL-1β and acute stress, with little attention on chronic stress or

IL-6 (Deak et al., 1997; Johnson et al., 2002; Nguyen et al., 1998; O'Connor et al., 2003; Pugh et al., 1999). IL-1β is only one element of the bi-directional, complex, inflamatory cascade that requires other cytokines, such as IL-6 (Maier, 2003; Maier & Watkins, 1998). Additionally, chronic social stress is a common experience that contributes to disease vulnerability and exacerbation, making it an important component of human health research.

Social Disruption Stress

Recent research using repeated exposure to SDR has focused on the variables of chronic stress and IL-6 (Johnson et al., 2004, 2006; Meagher et al., 2007; Quan et al., 2001). For example, Stark and colleagues (2002) showed that prior exposure to repeated sessions of SDR resulted in increases in IL-6 secretion in response to an immune challenge. SDR is a model of chronic social stress in which an older aggressive male is introduced into the home cage of three mice. In order to assert dominance over the home cage mice, the intruder displays typical aggressive behaviors such as digging, tail rattling, chasing, and attacking (Avitsur, Stark, & Sheridan 2001; Mackintosh, 1981). This procedure lasts for two hours and is generally given six times over seven nights (Avitsur, Stark & Sheridan 2001; Johnson et al., 2004; 2006; Meagher et al. 2007).

Previous research has shown that SDR increases circulating and central IL-6, exacerbates sickness behaviors induced by disease and endotoxic shock, and disrupts viral clearance (Avitsur, Stark, & Sheridan, 2001; Johnson et al., 2004, 2006; Meagher et al. 2007; Stark et al., 2002; Quan et al., 2001). Additionally, research has shown that central administration of IL-6 neutralizing antibody (AbTx) during the SDR period

prevents the stress-induced exacerbation of acute TMEV infection (Meagher et al., 2007). These findings suggest that an increase in central levels of IL-6 mediates the cross-sensitization of SDR induced cytokines and exacerbation of subsequent TMEV infection.

Given that social stress is a common human experience and that prior exposure to stress exacerbates both TMEV (Johnson et al., 2004, 2006; Meagher et al, 2007; Young et al., 2010) and MS (cf. Mohr et al., 2004), understanding IL-6 mediation of social stress induced sensitization and exacerbation of disease may lead to therapeutic interventions for the prevention and treatment of human diseases, such as multiple sclerosis (MS). Therefore, in this thesis we examined the necessity of IL-6 in SDR induced cross sensitization of TMEV pro-inflamatory cytokine release. We will discuss the natural disease course and symptoms of MS and TMEV, a virally initiated mouse model of MS, in the next section.

Multiple Sclerosis, Theiler's Virus, and the Viral and Stress Interaction Hypothesis

MS is an inflamatory autoimmune disease that causes white matter inflammation, immune activation with the increased secretion of CNS pro-inflamatory cytokines, and marked degeneration of the myelin sheath (Sospedra & Martin, 2005). Clinical symptomatology includes motor, sensory, and cognitive impairment as well as pain, fatigue, and depression. Research suggests that viral infection and stress may interact with genetic factors to increase susceptibility to the disease (Ackerman et al., 2002; Monteyne, Bureau, Brahic, 1997; Sospedra & Martin, 2005). Exposure to certain viruses, such as herpes simplex and Epstein-Barr, during adolescence have been

associated with later development of MS (Sospedra & Martin, 2005). Research also suggests that stress is linked with disease onset and exacerbation (Akerman et al., 1998, 2002; Mohr, 2004; Mohr and Pelletier, 2006; Meagher et al., 2007). Recent animal research with TMEV has shown a similar pattern of stress and viral infection interactions that determine disease severity (Johnson et al., 2004, 2006; Meagher et al., 2007; Sieve et al., 2004, 2006; Young et al., 2010).

Intracerebral infection with TMEV induces a biphasic disease process. After inoculation with TMEV, genetically susceptible strains of mice develop an acute infection characterized by ruffling, hunching, anhedonia, motor impairment, and CNS inflammation (Johnson et al. 2004, 2006; Meagher et al., 2007). Susceptible strains fail to clear the virus and develop a persistent infection of CNS-resident microglia and CNS-infiltrating macrophages that manifests with multiple sclerosis-like autoimmune and virus mediated demyelination within 3-5 months (Lipton 1975; Meagher and Welsh, 2009; Sieve et al., 2004).

Previous research from our laboratory has shown that repeated exposure to SDR prior to infection with TMEV causes exacerbation in both the acute and chronic phases of the disease. Stress exacerbated chronic phase symptoms including motor impairment, demyelination, and meningitis (Sieve el al., 2004, 2006; Young et al., 2010). Acutely, prior exposure to SDR increases TMEV induced inflammation in the spinal cord and brain, is associated with increases in circulating IL-6, and induces glucocorticoid resistance (GCR), a phenomenon whereby immune cells become insensitive to the anti-inflamatory effects of glucocorticoids (Johnson et al., 2004, 2006). Additionally, SDR

exacerbates acute motor impairment, cytokine associated sickness behaviors, and disrupts viral clearance. Research suggests that exacerbations of acute phase symptomatology generally predict exacerbations in chronic phase symptomatology, therefore this thesis will focus on acute phase disease (Johnson et al., 2006). Research has shown that IL-6 is necessary for SDR induced exacerbation of TMEV (Meagher et al., 2007). During these studies, researchers anecdotally noted individual differences in the immune and behavioral response to SDR. Because research has suggested that SDR may be mediated by social rank (Avitsur et al., 2007; Avitsur, Stark, and Sheridan 2001), we will discuss this factor in the next section.

Social Rank

Not all individuals display the same behavioral or physiological response when they encounter a stressor. Additionally, there are individuals that, when exposed to chronic stress, do not progress towards disease when challenged (Bartolomucci et al., 2005). Understanding of the causes of such individual differences and the consequences of this variability is needed to develop better treatment and prevention plans. Factors such as emotionality, obesity, childhood trauma, and social rank are possible modulators to explore (Avitsur et al., 2007; Avitsur, Startk, and Sheridan 2001; Flint et al., 1995; Locurto et al.; 2006; Pasquali et al., 1996).

Despite the genetically homogenous nature of inbred mice, experimenters observe significant variance within groups in a variety of research areas including learning, stress, activity, anxiety, and social interactions (Audet & Anisman 2009; Avitsur at al., 2007; Benton, Dalrymple-Alford, & Brain, 1980; Bartolomucci et al.,

2005; Fitchett, Barnard, & Cassaday. 2009; Flint et al., 1995; Locurto et al., 2006; Malloy et al., 2005). The two most cited modulating variables that explain such variance are social rank and emotionality/anxiety.

Anxiety, sometimes referred to as emotionality or reactivity, has been shown to account for some of the unexplained variance in many commonly used behavioral tests including open field activity, light/dark test, elevated plus maze spatial learning tasks, nose poke operant conditioning, and fear conditioning (Flint et al., 1995; Locurto et al.; 2006). While emotionality has been shown to be a good predictor of variance in activity and learning tasks, when studying stress and immunity, a wider variety of research points to social factors such as rank and rearing condition (Audet & Anisman, 2009; Avitsur et al., 2007; Avitsur, Stark, & Sheridan, 2001; Benton, Dalrymple-Alford, & Brain, 1980; Fauman, 1987; Ferrari et al., 1997; Haemishch, Voss, & Gartner, 1994; Merlot et al., 2004). For this reason, we will focus on social rank as a possible modulator for variance that is not explained by our previously established independent variables of SDR and TMEV infection.

Some research indicates that the inflamatory effects seen in SDR may be driven largely by one of the residents within a set of group housed mice, specifically the most subordinated mouse in the cage (Avitsur et al., 2007; Avitsur, Stark, and Sheridan, 2001). Avitsur, Stark, and Sheridan (2001) define social rank using a submissive ratio determined by dividing the time spent in submissive postures by the time being attacked by an intruding dominant mouse during the first 20 minutes of SDR. The mouse with the highest submissive ratio was labeled the most subordinate. They found that the most

subordinate animal was the only mouse to develop GCR in each cage. This finding would indicate that it is only this mouse that should show an exacerbated disease course after SDR.

However, the literature does not agree on the best way to test for social rank. Indeed, it is difficult to determine the construct validity of many tests of social rank, given that they do not always correlate with one another and vary greatly across laboratories, strains, and ages of mice (Audet & Anisman, 2009; Avitsur, Stark, and Sheridan, 2001; Bartolomucci et al., 2005; Benton, Dalrymple-Alford, & Brain, 1980; Fitchett, Barnard, & Cassaday, 2009; Lindzey, Winston, & Manosevitz, 1961; Merlot et al., 2004; Pereez et al., 2009). Therefore, we employed multiple tests of social rank to evaluate whether it determines the effect of SDR on acute TMEV infection. Next, we will introduce our primary dependent variables starting with the main behavioral readouts of SDR and acute TMEV: sickness behaviors.

Cytokines and Sickness Behaviors

When the immune system is activated by disease, such as TMEV, or chronic stress, such as SDR, macrophages and microglia are stimulated to release pro-inflamatory cytokines such as IL-1β, IL-6, and TNFα (Ackerman et al. 2002; Bluthè et al., 2000; Dantzer & Kelley, 2007; Johnson et al., 2006; Mohr & Pelletier, 2006; Nair & Bonneau, 2006; Quan et al., 2001). These pro-inflamatory cytokines are pyrogenic and trigger a coordinated set of changes known as sickness behaviors (Dantzer et al., 1999; Kansman, Parnet, & Dantzer, 2002; Kelley et al., 2003). Sickness behaviors, together with fever, reflect a centrally motivated and highly organized strategy of changes in the

body that help to fight illness (Dantzer et al., 1999; Konsman, Parnet, & Dantzer, 2002). Although sickness behaviors are a generally adaptive response to acute immune challenge, under conditions of chronic activation these inflamatory signals can be maladaptive and may lead to deleterious consequences. For example, short-term anorexia promotes adaptive diet selection, but long term anorexia can lead to wasting and significant body weight loss (Konsman, Parnet, & Dantzer, 2001). Some commonly observed sickness behaviors include: anorexia, anhedonia, fatigue, lack of interest in social behavior or personal grooming, hyperalgesia, and marked differences in sleep patterns (Bluthè et al., 2000; Dantzer et al., 1999; Goshen et al., 2008; Kansman, Parnet, & Dantzer, 2001; Kelley et al., 2003; Pollmacher et al., 2001).

Research has shown that pro-inflamatory cytokines are both necessary and sufficient to induce a number of sickness behaviors (Bluthè et al., 2000; Goshen et al., 2008; Merali et al., 2003). For instance, the deletion of IL-6 gene expression attenuates the depression of social exploration, activity, and body weight seen with administration of LPS and IL-1β (Bluthè et al., 2000). Furthermore, Merali and colleagues (2003) showed that a single interperitoneal injection of IL-1β was sufficient to induce anhedonia and anorexia.

Knowing that both illness and chronic stressors induce cytokine related sickness behaviors, it is not surprising that prior exposure to chronic stressors have been shown to exacerbate the onset and ongoing symptoms of illness in such diseases as TMEV infection and Multiple Sclerosis (Ackerman et al., 2002; Avitsur, Stark, & Sheridan, 2001; Avitsur et al. 2007; Meagher et al., 2007; Merlot et al., 2003; Quan et al., 2001).

Previous research in our laboratory has shown that prior exposure to SDR exacerbates both acute and chronic TMEV infection (Johnson et al., 2004, 2006; Meagher et al., 2007). This thesis will further our understanding of SDR and TMEV induced sickness behaviors by measuring anhedonia, motor activity, anorexia, and mechanical sensitivity. In the section that follows, we discuss the specific aims of this thesis.

Aims of This Study

Our previous research suggests a role for the central pro-inflamatory cytokine IL-6 in mediating the adverse effects of SDR on sickness/motor behaviors and inflammation during acute TMEV infection (Meagher et al., 2007); however, the exact mechanism(s) remain unclear. The goal of this thesis was to test the hypothesis that the adverse behavioral and neuroimmune effects of SDR on acute TMEV infection are mediated by the sensitization of cytokine expression. We hypothesized that repeated social stress would increase IL-6 mRNA expression in the brain, which, in turn, would exacerbate virus-induced cytokine expression and sickness behaviors/motor impairment during early infection. Specifically, we expected stress-induced increases in central IL-6 to mediate the adverse effects of SDR on acute TMEV infection through a cross-sensitization of virus-induced cytokine expression. We measured cytokine expression of mRNA for the pro-inflamatory cytokines IL-6 and IL-1β as well as CD11b, a widely used C3b-activated microglia marker.

Experiment 1 evaluated whether exposure to one or six sessions of SDR increased IL-6, IL-1β, and CD11b mRNA expression in the brain and the time course of these possible increases. In addition, this study tested the hypothesis that stress-induced

increases in cytokine expression were associated with the induction of mild stress-induced sickness behaviors. Based on our previous work (Meagher et al., 2007), we hypothesized that exposure to SDR would increase IL-6 mRNA expression in the CNS after six SDR sessions.

Furthermore, we predicted that SDR would exacerbate sickness behaviors when administered prior to infection with TMEV and that infected SDR subjects would show a greater increase in pro-inflamatory cytokine mRNA expression than controls. We also hypothesized that IL-6 was necessary for stress-induced exacerbation of TMEV infection. Experiment 2 was designed to test this hypothesis by administering an intracerebroventricular (ICV) infusion of neutralizing antibody to IL-6 (AbTx) concurrent with stress. We predicted AbTx during the stress exposure period would prevent SDR-induced exacerbation of acute TMEV infection. Other research indicates that stress (Frank et al., 2007; Nair and Bonneau, 2006; Sugama et al., 2007) and TMEV infection (Dal Canto and Vanderlugt, 2005) activate microglia. For this reason, we also tested for microglia activation with CD11b following stress and infection.

As discussed previously, another possible modulator of stress-induced exacerbation of TMEV infection may be social rank. To test this modulator we used Avitsur, Stark and Sheridan's (2001) scoring method to assign a social rank to the mice. We then re-analyzed behavioral and biological data from Experiment 2 using social rank to determine if we observed any effect of social rank in our paradigm. Avitsur, Stark, and Sheridan's (2001) operational definition of social rank is, however, limited in scope and only looks at within cage interactions in response to SDR. Therefore, we tested

social rank using a variety of methods that the independent variable SDR, but instead on the interactions of the home cage mice, to determine if social rank explains some of the variability in response to SDR. It is important to evaluate this issue because, if only one mouse within a cage exhibited an SDR-induced exacerbation of behavior and inflammation, then the other mice might have masked some effects. Additionally, it would justify the use of social subordination as a mediating co-variable, thereby increasing the power of our experimental tests. Furthermore, this study allowed us to determine if the SDR procedure would be effective when mice were housed two per cage as opposed to three. The central hypothesis of this thesis was that SDR would cause an up-regulation of CNS inflammation which would exacerbate TMEV infection and that this exacerbation was mediated by stress-induced CNS IL-6 expression and modulated by social rank. In the next section, we will provide a detailed overview of the methods used.

GENERAL METHOD

Subjects

Male Balb/cJ mice (Jackson Labs, Bar Harbor, ME) were individually housed upon arrival (mice in Experiment 3 were group housed upon arrival). Animals were between 22 and 24 days old at arrival (Experiment 1 mice were 4 weeks old). In those experiments requiring surgery the mice were allowed to recover for three days. The mice were housed three per cage (for Experiment 3b mice will be housed two per cage), and counterbalanced for weight across cages and groups. Mice were then maintained on a 12 hour light/dark cycle (lights on at 05:00 h) with *ad libitum* access to food and water. Dominants were retired Balb/cJ male breeders aged 6-12 months. They were individually housed, screened, and picked for aggressive behaviors by placing them in the home cage of another dominant mouse and vice-versa.

Independent Variables

Social Disruption Stress (SDR). Dominants were introduced into the experimental mouse home cage at the onset of the dark cycle for a period of two hours. SDR occurred for three consecutive sessions, then one night off, followed by three additional consecutive sessions, for a total of six SDR sessions. Each cage of stressed mice was exposed to a new intruder for each of the six sessions. SDR sessions were monitored and recorded to ensure that the intruder attacked the residents and that the residents demonstrated submissive behaviors. If intruders did not attack within 10 minutes of the start of a session, they were replaced and the session continued for the remaining 2 hours. Intruders were selected using a dominance test in which didactic

encounters between all dominant intruders were observed. Only the most aggressive intruders from a group (those displaying the most aggressive behavior and/or initiating the most fights) were chosen as dominant intruders for SDR. It should be noted however that this procedure was completed more rigorously for Experiment 3 than for Experiments 1 or 2 in order to correct for a laboratory drift in methodology.

ICV Surgery. Mice were anesthetized with isoflurane gas (2-5%). Their heads were shaved with an electric trimmer and petroleum jelly was applied to their eyes to prevent drying. The mice were then placed in a mouse adapted sterotaxic device. The skull was exposed by a longitudinal incision along the midline of the skull. Using a dermal drill, a cannulation hole was drilled at +1 mm lateral to bregma and -0.4 mm rostral to bregma over the left lateral ventricle. A guide cannula (33g, pre-cut to a depth of 1.75 mm) was implanted and secured with superglue. Mice were then put back into their individual cages with Tylenol water (325 mg/2 L) softened food and given Tylenol water to drink. They recovered for 3 days prior to group housing.

ICV Injections. Two hours prior to the start of SDR mice in Experiment 2 received an injection of either neutralizing antibody or vehicle. Administration was through an indwelling cannula and 2 µl of solution was infused over 2 minutes followed by a 30 second delay to prevent removal of the solution with the removal of the guide cannula. This method of administration was achieved using a 25 µl Hamilton syringe, plastic tubing, and a guide cannula all fitted to a regulated injection pump.

Infection with TMEV. The BeAn strain of Theiler's virus (obtained from Dr. H.L. Lipton, Departmetn fo Microbiology-Immunology, University of Illinois, Chicago,

IL.) was initially propagated in lung tumor (L2) cells. In applicable experiments, mice were anesthetized with isoflurane (Vecco Inc., St. Joseph, MS) and inoculated into the right mid-parietal cortex (1.5 mmdepth) with $5X10^4$ pfu of TMEV in 20-µL volume two hours after the last SDR session.

Tests of Dependent Variable Sickness Behaviors

SDR Related Wounding. To systematically assess the degree of wounding induced by SDR, we used a measure adapted from Merlot et al. (2003). Before each session the SDR and Non-SDR mice were assigned a score ranging from 1 to 4. The score was as follows: 1 (fur well groomed and polished), 1.5 (fur not so well polished, might look a bit ruffled or dirty), 2 (a small number of marks or bristling of the fur), 2.5(one small bite), 3 (numerous marks/bites with bristling of the fur), 4 (one or more visible wounds where the fur was obviously disrupted. Directly after each SDR session mice were examined closely under a red light and any visible wounds were noted (gross score of bites or severe ruffling).

Sucrose Preference. We used sucrose preference to measure anhedonia. Mice were provided with a 2% sucrose water bottle and a tap water bottle 4 days prior to the start of SDR. The position of the sucrose water bottle and tap water bottle was switched daily to prevent any place preference. Sucrose preference was calculated by dividing the intake of the sucrose solution by the total fluid intake. Cages that had 60% or more preference prior to experimental manipulation were included in analysis. Due to the nature of this test, all data is per cage, not per animal.

Food Consumption. Food was weighed daily and the amount of food consumed per cage was determined and used for analysis. Due to the nature of this test, all data is per cage, not per animal.

Body Weight. Mice were weighed at 9:00 am every morning using a scale sensitive to 0.01 grams and amount of weight gained or lost was calculated and used for analysis.

Hind Limb Impairment (HLI). Acute infection with TMEV causes distinct hind limb impairment in the Balbc/J strain of mouse that consists of weakness and paresis in the hind limbs. Hind limb impairment was assessed in experiments that include infection on days 1, 4, and 7 with a baseline at day -1. Raters were blind to the subject's experimental conditions. Mice were given a 0-5 HLI score and the numbers were: 0=healthy, 1=slight weakness in grip, 2=clear weakness in grip, 3=slight paralysis, 4=moderate paralysis, 5=complete paralysis with muscle tone, 6=complete paralysis with no tone.

Clinical Score. Acute infection with TMEV causes clinical score in mice that behaviorally manifests as hunching of the spine and ruffling of the fur. Clinical score was assessed in experiments that include infection on days 1, 4, and 7 with a baseline at day -1. Raters were blind to the subject's experimental conditions. Mice were given an clinical numeric score based on the following: 0-6 for level of ruffling with a 0 being smooth fur, 6 being very oily, clumped fur over 100% of body; and a 0-6 for level of hunching with 0 being no hunching and 6 having a sharp, high bump between the shoulder blades and rear hind quarters abnormally dropped low to the ground.

Basso, Beattie, Brenahan Locomotor Rating Scale for Mice (Mouse BBB). In order to further assess hind limb impairment in the third experiment, we employed the mouse BBB scale as outlined in Basso et al. (2006) in which hindlimb stepping was assessed while viewing subjects in an open field. Hind limb stepping with the mouse BBB scale was assessed in Experiment 3 on days 1, 4, and 7 with a baseline at day -1. Each experimental mouse was placed in an open field and scored by a blind experimenter using the mouse BBB scale. Mice were given a score from 0 to 9 based on the extent and quality of ankle movement, stepping, and coordination (see Appendix).

Activity Monitoring. Mice were habituated to one of six open field chambers for one hour prior to testing. They were tested in a dark room between 15:00 and 16:00 h with white noise present to prevent auditory disturbances. Each testing period lasted for 30 minutes and measured a variety of activities including vertical activity, horizontal activity, rest, movement, center entries, and center time. Center time and entries were used as a measure of anxiety while movement, rest, vertical activity, and horizontal activity were used as measures of sickness.

Mechanical Sensitivity. Mice were placed in individual plastic test chambers on an elevated screen mesh floor so that the plantar surface of hind paws could be reached from beneath. Mechanical threshold was determined using the Von Frey filament test by administering filaments from 0.008 to 4.0 grams in ascending and descending order (A-B, B-A, A-B).

Tests of Social Rank

Sexual Preference Test. In Experiment 3 we adapted a female sexual preference test from Avitsur, Pollak, and Yirmiya (1997). Male mice were put in the goal box of a T-maze behind a perforated door and tested in a dichotomous manner so that each mouse was paired against each of its cage mates. An in estrous female mouse was placed in the start box and allowed time to choose which male she prefers by smell and sight. The male that she spends the most time near will be deemed the dominant of the pair, because female mice tend to prefer the most dominant smelling male mouse out of a group. This appears to be due to specific major urinary proteins that bind to pheromones and increase the longevity of secreted signals in the urine of dominant males (Hurst, 2009; Mossman and Drikamer, 1996). Females were pushed into super ovulation (procedure from Jackson Laboratories) by administering 5.0IU of pregnant mare serum gonadrotropin (PMS) and then 48 hours later 5.0IU of human chorionic gonadotropin (HCG) via an interperitoneal injection. These treatments are designed to push mice into estrous about 12 hours after HCG administration (Jackson Laboratories).

Resident Intruder Test. In Experiment 3 a mouse one week younger than experimental mice was introduced into the home cage of experimental subjects for 15 minutes (Avitsur et al., 2007). These sessions were scored for social exploration of the intruder, attacks on the intruder and/or the other cage resident, and submissive behavior. The dominant mouse was defined as the resident that exhibited the highest duration of aggression towards the intruder or the other cage resident and/or the lowest level of submissive behavior. If no aggression is displayed then the dominant mouse was deemed

the resident that had the highest duration of social exploration of the intruder. If the social exploration difference between the residents is less than 5%, then the social status was undefined. We also explored ratios of aggressive behavior.

Food Competition Test. After 12 hours of food deprivation the whole group was transferred into the test area (Merlot et al., 2004). The test was completed at two time points in Experiment 3. The test was performed through analysis of didactic encounters between cage mates in the test area. Mice were competing for a small piece of vanilla cookie. An index (X) ranging from 1 to 5 will be calculated from resultants of pair comparisons: $X=(W-L+N+1)/2$, where W is the number of confrontations the subject won, L is the number of confrontations the subject lost and N is the group size. A given subject was identified as a High Ranker when $X>4$ (i.e. monopolizing the pellet in all or almost all encounters, and a low ranker $X<2$ (i.e. never or almost never having access to the food pellet).

SDR Dominance Scoring. SDR was videotaped and scored for subordination (Avitsur, Stark, and Sheridan, 2001). A submissive ratio was determined based on aggressive attacks and submissive responses during the first 20 minutes of the first session of SDR. The subordinate mouse was defined as the one with the highest submissive ratio. Other ratios of behavior were also explored. Mice were scored individually for the duration of aggressive attacks exhibited by intruder toward each resident, including mount, bite, and chase with physical contact. They were scored for duration of submissive responses exhibited by the resident including standing on hind

limbs with the ventral body surface directed toward the intruder and forelegs raised off of the ground.

Tissue Preparations

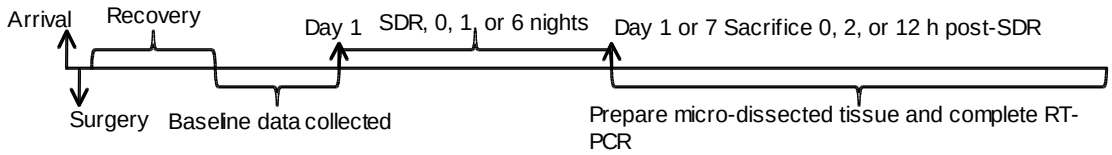
Sacrifices. Mice were deeply anesthetized with pentobarbital or beuthanasia and then bled from the brachial artery (for sera collection) or via a cardiac puncture with an EDTA prepared syringe (for plasma collection). Mice were then perfused with 10mL of RNAse free water (for Experiment 1 and two) or RNAse free PBS (for Experiment 3). Brains and spinal cords were taken for RT-PCR CNS inflammation assessment. The brain was cut in half (Experiment 1 hypothalamus, hippocampus, and cortex were micro-dissected out) for RT-PCR analysis. Tissue and blood samples were stored at -80ºC until time of analysis.

Real Time-PCR. Tissue was thawed and RNA extracted in QIAzol using either the RNeasy (QIAGEN) midi (half brain) or mini (spinal cord and specific brain regions) QIAzol RNAeasy kits per manufactures instructions. After assessment for RNA purity and quality, reverse transcriptase was achieved via the high capacity RNA to DNA kit (Applied Biosystems). RT-PCR was completed using bought TaqMan probes and primers (Applied Biosystems) to IL-6, IL-1β, and CD11b.

Data Analysis

All data was analyzed using ANOVAs, Bonferroni t-tests, linear hierarchical regressions, correlations, or Kruskal-Wallis analysis. When necessary, Tukey's post hoc tests were utilized Welch's correction was employed to correct for violation of unequal variance. A p value of 0.05 or less was considered significant.

EXPERIMENT 1: DOES SDR INDUCE AN INCREASE IN PRO-INFLAMATORY CYTOKINES IN THE CNS?



CNS inflammation in response to a stressor is dependent upon the type and timing of the stressor, what brain areas are assayed, and what cytokines are measured (Deak et al., 2005; Johnson et al., 2004, 2006; Meagher et al., 2007; Quan et al., 2001). Many studies have focused on acute stressors and/or the pro-inflamatory cytokine IL-1β, with little attention on the effects of IL-6 and repeated stressors. Recent research though has found that after 6 sessions of SDR, protein levels of IL-6 are high in the CNS of adolescent male mice (Meagher et al., 2007). To describe the mechanism of action behind these findings, we need to systematically describe the time course of SDR induced increases in IL-6. Research has also shown that IL-6 may control the effects of IL-β upon HPA axis activation, Experiment 1 also measured the time course of SDR induced IL-β (Matta, Weatherbee, & Sharp, 1992, Perlstein et al., 1993; Zhou et al., 1993). Additionally, research has shown that glial activation is also involved in the inflamatory response to TMEV (Mi et al. 2006; Sato et al, 1997), so if SDR increases microglia activation, it is another possible source of SDR induced exacerbation. To explore this theory we will measure microgila activation using the marker CD11b. We expect that after six sessions of SDR we will see increases in mRNA expression of IL-6, IL-1β, and CDllb. Because Merlot and colleges (2003) found that peripheral IL-1β is increased following one session of SDR, we expect that CNS levels may follow suit. We

do not however expect to see increases in the CNS levels of IL-6 and microglia activation after only one session of SDR. Due to the potential for cytokine related sickness behaviors we will characterize these throughout this experiment.

Procedure

We examined the effects of social disruption stress and timing on CNS inflammation using a 3 (SDR 0, 1, 6 session) x 3 (0, 2, 12 h sacrifice post-SDR) design with finial N as follows; SDR 0=17, SDR 1=15, SDR 6=18. Sample sizes are uneven due to attrition. Upon arrival, mice underwent surgery to implant an intracerebroventricular cannula and were given three days to recover. After recovery the mice were group housed for 3 days. During this time baseline data was collected for body weight, sucrose preference, food consumption, and mechanical sensitivity. After the third day of group housing, the mice underwent SDR for either 0, 1, or 6 sessions. In order to characterize SDR induced sickness behaviors we measured body weight, sucrose preference, food consumption and mechanical sensitivity throughout the stress exposure period. Three sacrifice time points after SDR (0, 2, and 12) were utilized to track the time course of CNS inflammation. At time of sacrifice the hippocampus was micro-dissected out of the brain and later used in RT-PCR analysis of IL-6, IL-1β, and CD11b. We expectd that mice receiving six sessions of SDR will show elevated levels of inflammation in the CNS but that animals receiving no or one session of SDR would not show elevated CNS inflammation except a possible up-regulation in IL-β.

Results

Repeated measures ANOVA tests revealed that behavioral measures were not statistically significant across groups ($p>0.05$) for Experiment 1 (see Figures 1 & 2). We did observe that there was a significant effect of time on mechanical sensitivity, $F(6,1)=50.897$, $p<0.05$, body temperature, $F(6,1)=3.446$, $p<0.05$, food consumption, $F(6,1)=9.431$, $p<0.05$ and body weight $F(6,1)=2.779$, $p<0.05$. Additionally, we see a systematic pattern of results for inflamatory mRNA expression in hippocampus. Two way ANOVAs revealed a significant main effect of SDR for IL-6 mRNA expression in hippocampus, $F(2,41)=20.487$, $p<0.05$ (see Figure 3). Post hoc analysis revealed IL-6 is significantly up-regulated in mice that received 6 SDR sessions but not in mice that received only 1 session of SDR or those that received 0 sessions, suggesting that 6 sessions of SDR sensitizes IL-6 mRNA expression in the hippocampus (see Figure 3a).

A two-way ANOVA of IL-1β revealed a significant effect of time of sacrifice, $F(2,40)=11.263$, $p<0.05$, and a significant interaction of SDR condition by time of sacrifice, $F(4,40)=11.034$, $p<0.05$. Post hoc analysis revealed that IL-1β mRNA expression is up-regulated at 12 hours after the 1st session of SDR suggesting that IL-1β mRNA expression from stress is habituating over time, with a robust expression after 1 session of SDR that is no longer seen after 6 sessions of SDR (see Figure 3b).

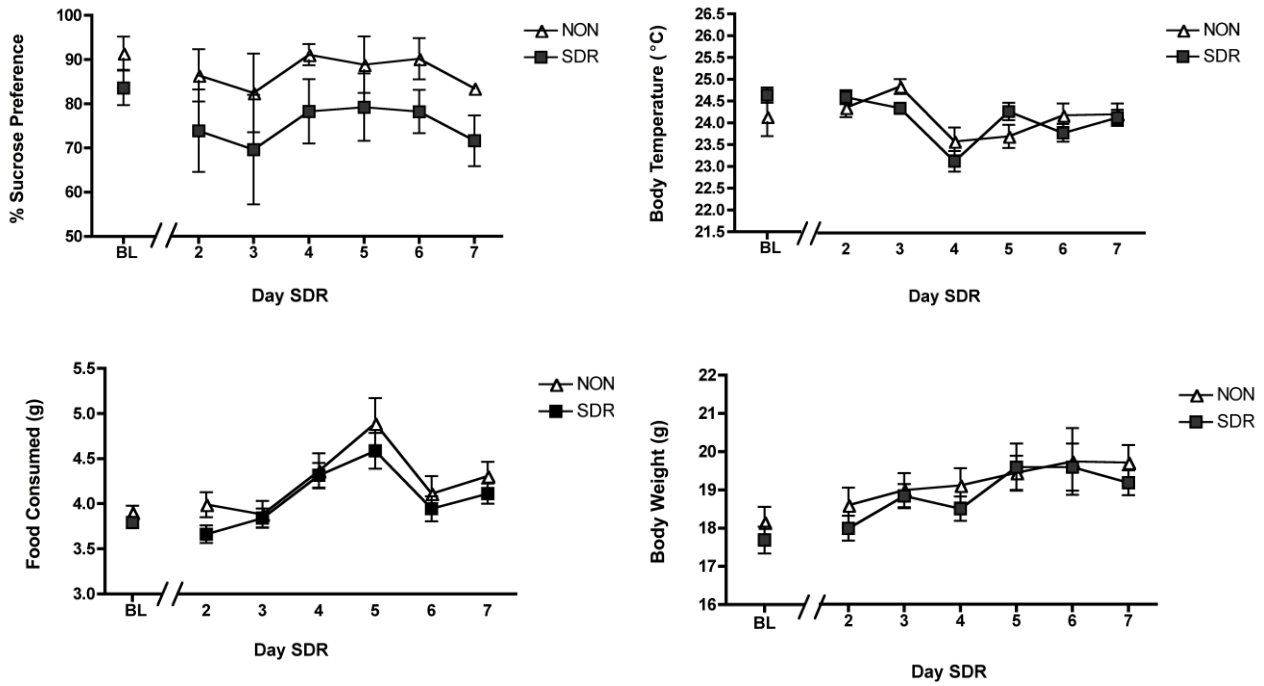


Figure 1. The impact of social disruption stress (SDR) on sickness behaviors. Measures of sickness syndrome are displayed over seven days of stress, including alterations in sucrose preference, body temperature, food consumption and body weight. There are no statistically significant differences between SDR and Non SDR groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON) and social disruption session (SDR).

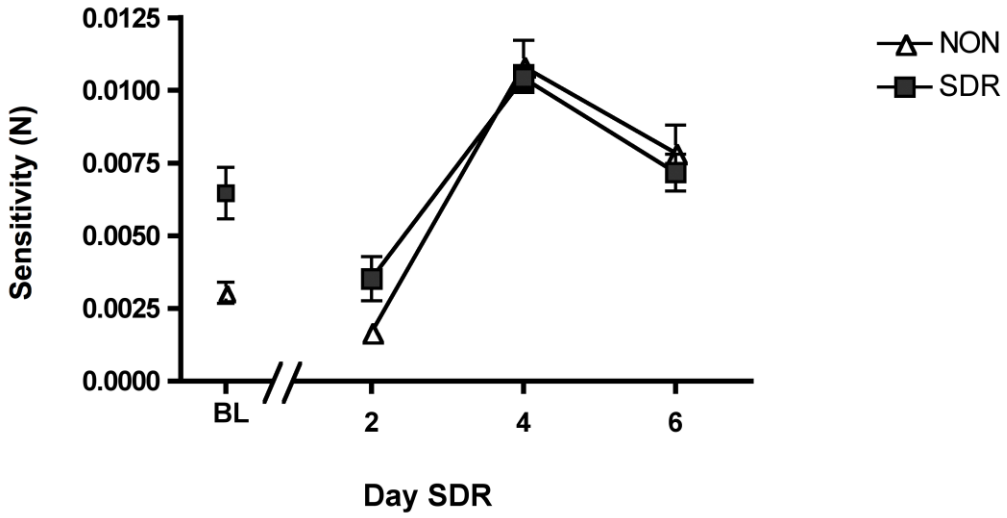


Figure 2. The impact of SDR on mechanical sensitivity. Data collected during SDR indicated that SDR does not have a significant effect on mechanical thresholds. Abbreviations for the experimental treatments are as follows: Non-stressed (NON) and social disruption (SDR).

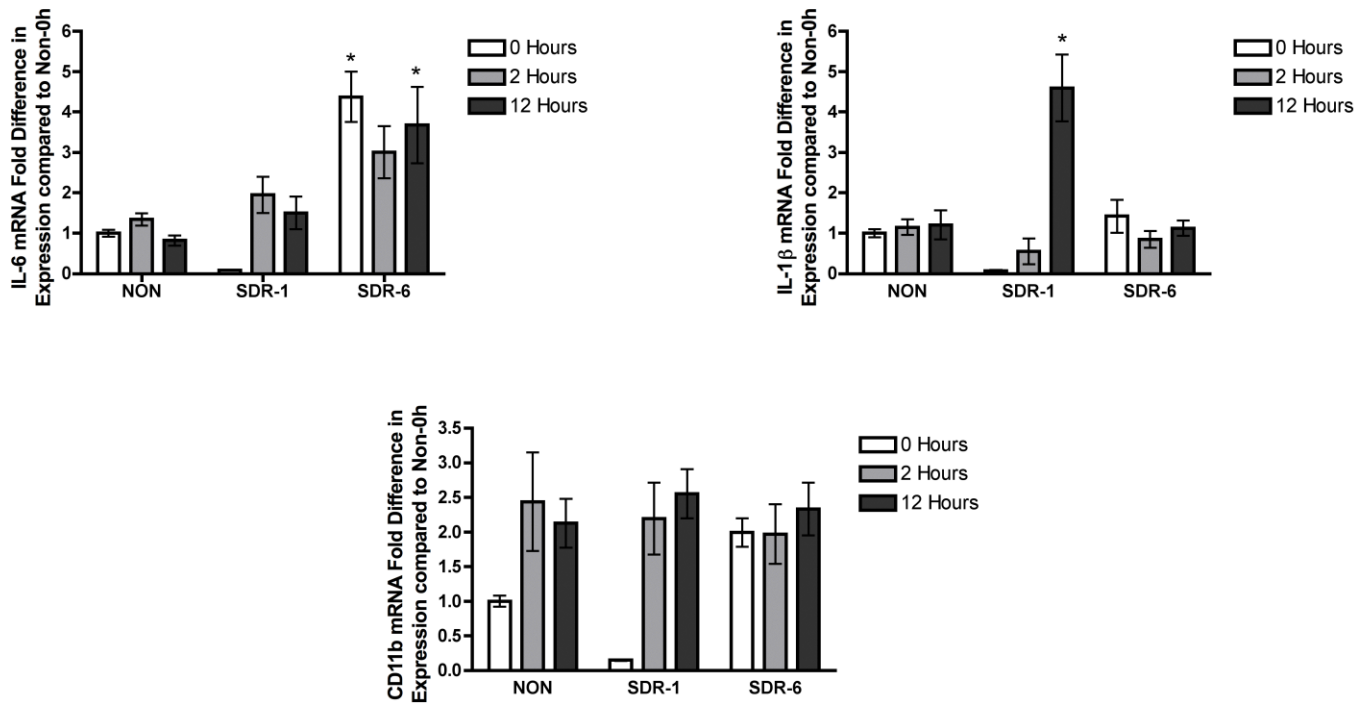


Figure 3. The impact of repeated SDR sessions on hippocampus inflamatory tone. SDR increased mRNA expression above Non-SDR control levels: a) IL-6 is increased 2 and 12 hours after the 1st SDR session, and at 0, 2, and 12 hours after the 6th SDR session, b) IL-1β is increased 12 hours after the 1st SDR session, and c) CD11b is decreased immediately following the 1st SDR session thereafter, CD11b is elevated at 2 and 12 hours in all three conditions. Asterisks indicate significant post hoc differences between groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON), social disruption session 1 (SDR-1), social disruption session 6 (SDR-6).

A two-way ANOVA revealed a significant main effect of time for CD11b mRNA expression, $F(2,41)=7.718$, $p<0.05$. *Post hoc* analysis revealed that CD11b mRNA expression in the hippocampus is significantly decreased immediately following the first session of SDR, and is then up-regulated for all groups at 2 and 12 hours post SDR, suggesting that the results seen for microglia activation may be reflecting a nonspecific circadian elevation and not necessarily a SDR induced CNS inflammation (see Figure 3c). Please note that while activity monitoring was completed for this experiment, the results were aberrant and therefore not reported here.

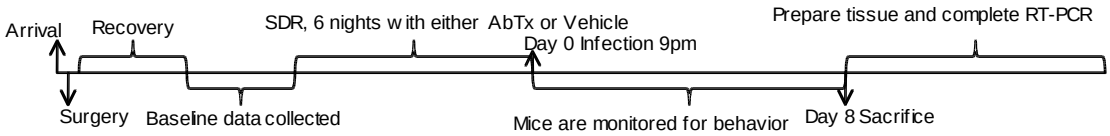
Discussion

CNS inflammation was observed following both the 1st session (IL-1 β at the 12 hour following SDR) and the 6th session of SDR (IL-6 at 0,2, and 12 hours post SDR). These results implicate the up-regulation of IL-6 as a possible mechanism for the observed SDR induced exacerbation of acute TMEV (Johnson et al., 2004, 2006; Meagher et al., 2007). Additionally they support the theory of a priming effect resulting from 6 sessions of SDR. We would expect mice given an immune challenge after 6 sessions of SDR would have a stronger behavioral and inflamatory immune reaction than one given the same immune challenge after 0 or 1 session of SDR. We believe that the effect of SDR on IL-1 β levels is habituating over time, after one session of SDR there is a robust response, whereas by the 6th session of SDR there is no longer a response.

Despite the elevations in CNS inflammation, neither 1 nor 6 sessions of SDR induced observable sickness behaviors. Either these levels of CNS inflammation are too

low to induce sickness or the measures used in this experiment were not sensitive enough to pick up on low levels of sickness behaviors. We may want to test cognitive impairment or learning in future studies as this type of challenge may be more sensitive to lower levels of inflammation. Although general increases in body weight and food consumption over time were observed, these increases are attributable to the natural growth and appetite of adolescent mice. Activity monitoring is routinely done in a different part of the laboratory than most other behavioral testing. During this experiment a laboratory in close proximity was using a stressful scent that permeated the surrounding areas periodically. This and possible drifts in procedure of laboratory set up or data acquisition could be responsible for the aberrant activity monitoring data.

EXPERIMENT 2: DOES PRIOR EXPOSURE TO SOCIAL STRESS SENSITIZE VIRUS-INDUCED IL-6 DURING ACUTE THEILER'S VIRUS INFECTION?



Previously we have found that SDR prior to acute infection with TMEV induces increases in sickness behaviors associated with acute TMEV infection (Johnson et al. 2004, 2006; Meagher et al., 2007). We have also found that SDR prior to infection increases CNS protein levels of the pro-inflamatory cytokine IL-6 prior to infection. From these and the findings from Experiment 1 of this thesis, we suspect that this increase in inflamatory tone primes the CNS and causes higher than normal infection levels of sickness behaviors and CNS inflammation with a subsequent TMEV challenge. We also suspect that increased levels of IL-6 may be the underlying mechanism behind this stress-induced exacerbation. Because social stress and TMEV independently increase CNS cytokine expression, we expect that prior exposure to SDR will amplify central inflammation in response to TMEV. Additionally, SDR-induced IL-6 is a possible mechanism behind SDR exacerbation of TMEV infection; therefore we expect that central infusion of IL-6 neutralizing antibody (AbTx) will prevent SDR amplification of central inflammation and sickness in response to TMEV.

Procedure

To examine whether SDR-induced increases in IL-6 sensitize the inflamatory and sickness response to Theiler's virus infection we used a 2(SDR x Non SDR) by 2(AbTx x Vehicle) design with 12 subjects per condition (N=48). Upon arrival mice

underwent cannulation surgery and recovered for three days. After recovery the mice were group housed, without manipulation, for three additional days. During this time baseline data were collected for body weight, sucrose preference, food consumption, activity, mechanical sensitivity, hind limb impairment, and clinical score. These behavioral measures were also collected during SDR and post-infection. Next, mice in the SDR condition underwent SDR for six nights starting at 5pm. Prior to SDR each day at 2:00 pm, all mice received an infusion of either IL-6 neutralizing antibody (AbTx) or vehicle (VEH or IgG). Two hours after the last session of SDR all mice were inoculated with TMEV. Mice were monitored for behavior and sickness for eight days post infection and then sacrificed. CNS tissue was collected and later analyzed using RT-PCR for mRNA expression of IL-6, IL-1β, and CD11b. We expected that SDR would exacerbate infection and induce increased levels of CNS inflammation and that infusion with IL-6 AbTx would prevent this exacerbation.

Results

A MANOVA revealed a significant interaction between SDR and AbTx for the composite variable of mRNA expression of IL-6, IL1β, and CD11b in brains at day 8 for Experiment 2, $F(1,40)=4.676$, $p<0,05$. Further Bonferroni *t*-test planned comparisons revealed that SDR resulted in a significant increase in IL-6 and IL-1β and that administration of AbTx prevented this effect. While not statistically significant, the pattern of results for CD11b is similar, indicating that microglia activation might be involved in the SDR exacerbated CNS inflamatory response to Theiler's virus (see Figure 4).

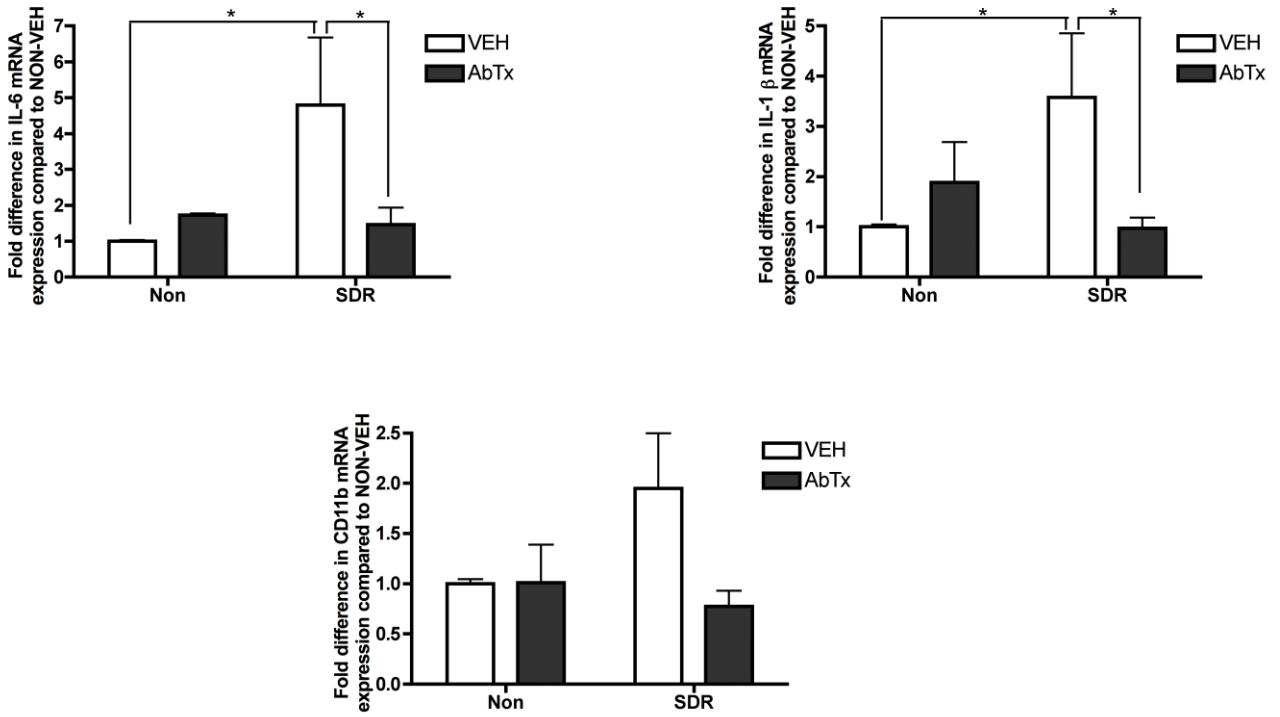


Figure 4. Social stress prior to infection resulted in increased virus-induced IL-6 and IL-1β cytokine expression in half brain, which was prevented by central infusion of the IL-6 neutralizing antibody (AbTx) during the stress exposure period. While not statistically significant, the pattern of results for CD11b is similar. Asterisks indicate significant *post hoc* differences between groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON), social disruption (SDR), IL-6 neutralizing antibody treatment (AbTx), and vehicle (VEH).

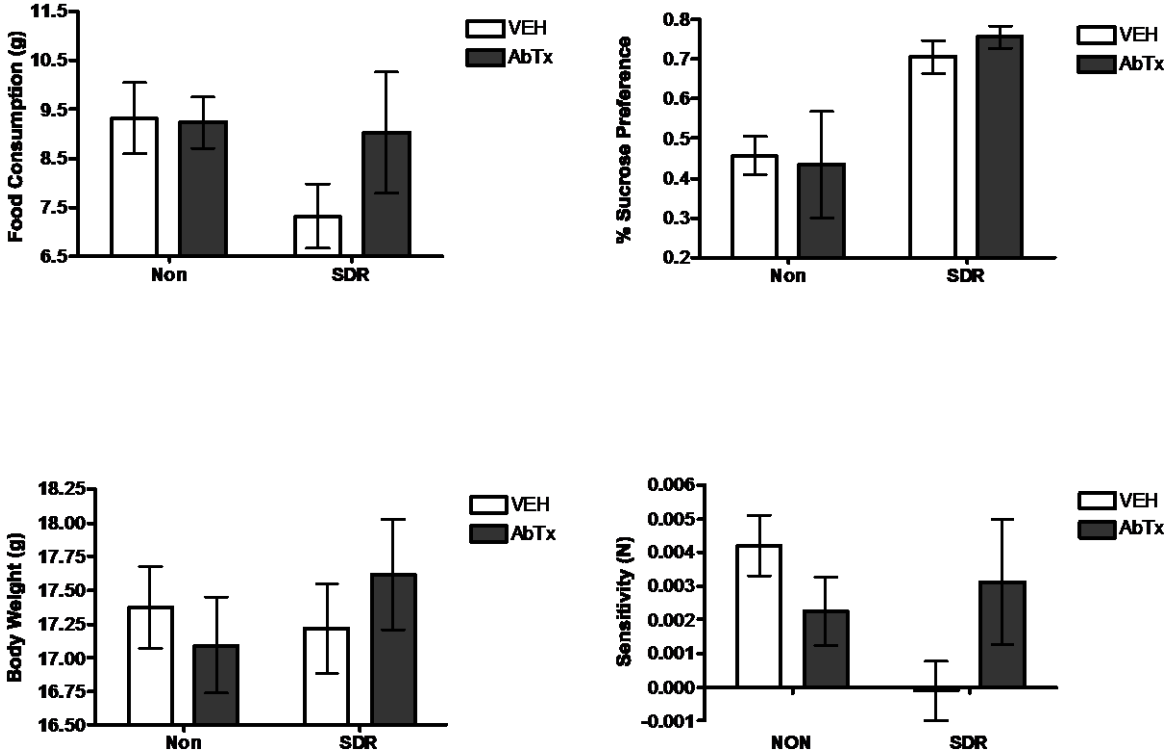


Figure 5. Impact of IL-6 neutralizing antibody (IL-6 AbTx) and SDR on sickness behaviors during acute TMEV infection. The effect of SDR on mechanical sensitivity thresholds (day 7 post infection) and food consumption (day 1 post infection) was reversed by administration of the neutralizing antibody during the stress exposure period. However, it did not alter sucrose preference or body weight. Asterisks indicate significant post hoc differences between groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON), social disruption (SDR), IL-6 neutralizing antibody treatment (AbTx), and vehicle (VEH).

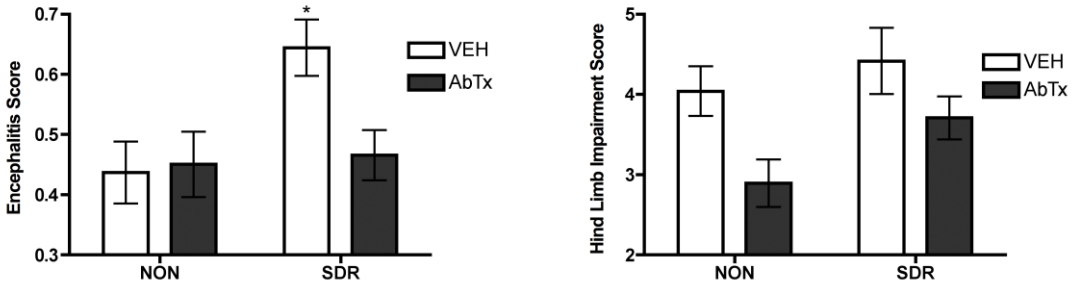


Figure 6. Impact of IL-6 neutralizing antibody (IL-6 AbTx) and SDR on clinical scores and hind limb impairment. SDR increased clinical scores in the vehicle treated mice and importantly, infusion with IL-6 AbTx prevented this stress induced exacerbation. However, there were no statistically significant differences in hind limb impairment scores between groups. Asterisks indicate significant *post hoc* differences between groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON), social disruption (SDR), IL-6 neutralizing antibody treatment (AbTx), and vehicle (VEH).

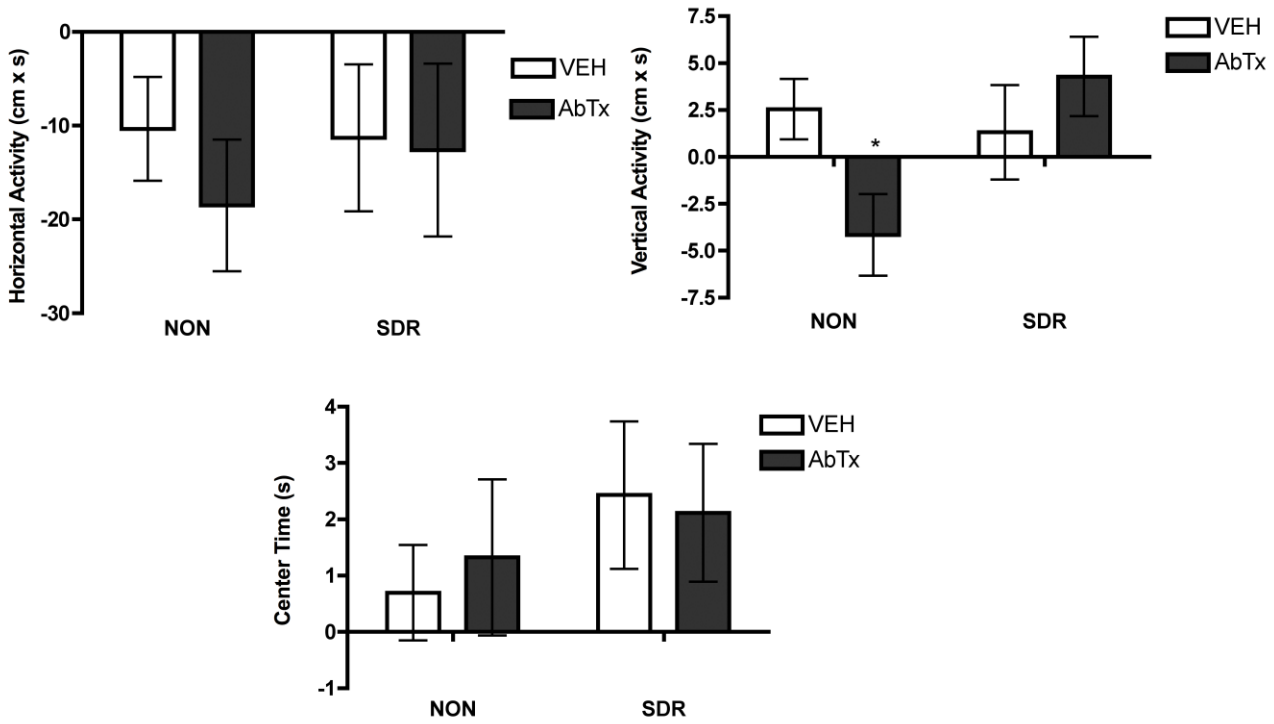


Figure 7. Horizontal and vertical activities were used as a measure of lethargy, whereas center time was used as a measure of anxiety. There was no effect of SDR or AbTx on horizontal activity or center time. Although the effect of SDR on vertical activity trends toward AbTx preventing SDR induced lethargy, it was not significant. Unexpectedly, IL-6 AbTx induced a decrease in vertical activity for NON SDR mice. Asterisks indicate significant post hoc differences between groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON), social disruption (SDR), IL-6 neutralizing antibody treatment (AbTx), and vehicle (VEH).

Even though some behavioral measures (food consumption, mechanical sensitivity, and motor impairment) approached significance and were patterned in the predicted direction, a series of two-way ANOVAs tests revealed that most behavioral measures were not statistically significant ($p>0.05$) for Experiment 2 (see Figures 5, 6, & 7). Two-way ANOVAs revealed a statistically significant SDR x AbTx interaction and *post hoc* test showed that SDR animals receiving vehicle scored significantly worse on clinical score than all other groups, $F(1, 44)=4.229$, $p<0.05$ (see Figure 5). Additional two-way ANOVAs revealed a significant SDR x AbTx interaction for change from baseline day 1 vertical activity, $F(1, 44)=4.719$, $p<0.05$ (see Figure 6).

Discussion

As predicted, chronic stress exacerbated subsequent TMEV infection. Additionally, when IL-6 AbTx was administered concurrently with stress, this exacerbation was blocked. Importantly this pattern of results was observed in CNS inflammation. Also, whereas only one behavioral measure was significant in the predicted pattern many measures showed a similar pattern. Taken together, these results support the theory that IL-6 is necessary for the priming effect of SDR on subsequent immune challenge with TMEV.

EXPERIMENT 3: IS SOCIAL STRESS INDUCED EXACERBATION OF ACTUE THEILER'S VIRUS INFECTION MODULATED BY PREVIOUSLY ESTABLISHED SOCIAL RANK?

Research has shown that social rank may be a modulating factor in how male mice react to social stress in that more subordinate mice develop higher levels of GCR than less dominant or subdominant mice (Avitsur, Stark, and Seridan, 2001). Indeed, we have observed individual variability in our data when testing the effect of SDR on acute TMEV infection and suspect that the moderating variable might be previously established social rank. In order to answer the question of whether SDR induced exacerbation of acute Theiler's virus infection is mediated by previously established social rank, we looked at social rank first with previously collected data and then with a more extensive experiment.

Experiment 3a Procedure

The first session of SDR from Experiment 2 was taped and scored using a method adapted from Avitsur, Stark, and Sheridan (2001). They taped the first 20 minutes of SDR and scored each experimental mouse for a subordinate ratio. Mice that received social disruption stress were blindly scored and assigned a social rank. To determine whether social rank may account for individual differences in the impact of SDR on TMEV infection, the behavioral and immunological data previously collected during Experiment 2 was reanalyzed entering rank scores.

Results

Only data from SDR mice are presented due to the the nature of the scoring system, and only IgG treated mice. We ran analyses on SDR AbTx mice and, not surprisingly, no significant trends or differences were revealed. We thush came to a data driven decision not to report the results as due to our previous hypothesis and findings. Data collected using the social ranks calculated from the SDR videos for Experiment 2 revealed some interesting patterns. We have run Kruskal-Wallis ANOVAs on all of these variables. Kruskal- Wallis ANOVA is a Chi-square based, non-parametric ANOVA. It is an appropriate test because it is not as affected by the limited range of data, and the independent variable is ranked data. None of these tests showed significant differences between ranks, probably due to the small N of 4 per group, though some were approaching significance. Regardless of statistically significant differences, we observed a trend of the dominant mouse in the cage having higher levels of CNS inflammation following SDR and infection, as seen in mRNA expression of IL-6, IL-1β, and CD11b. Additionally we see a trend of the most submissive mouse in a cage showing the strongest behavioral following SDR and infection. This trend was observed in percent body weight loss and horizontal activity (see Figure 8).

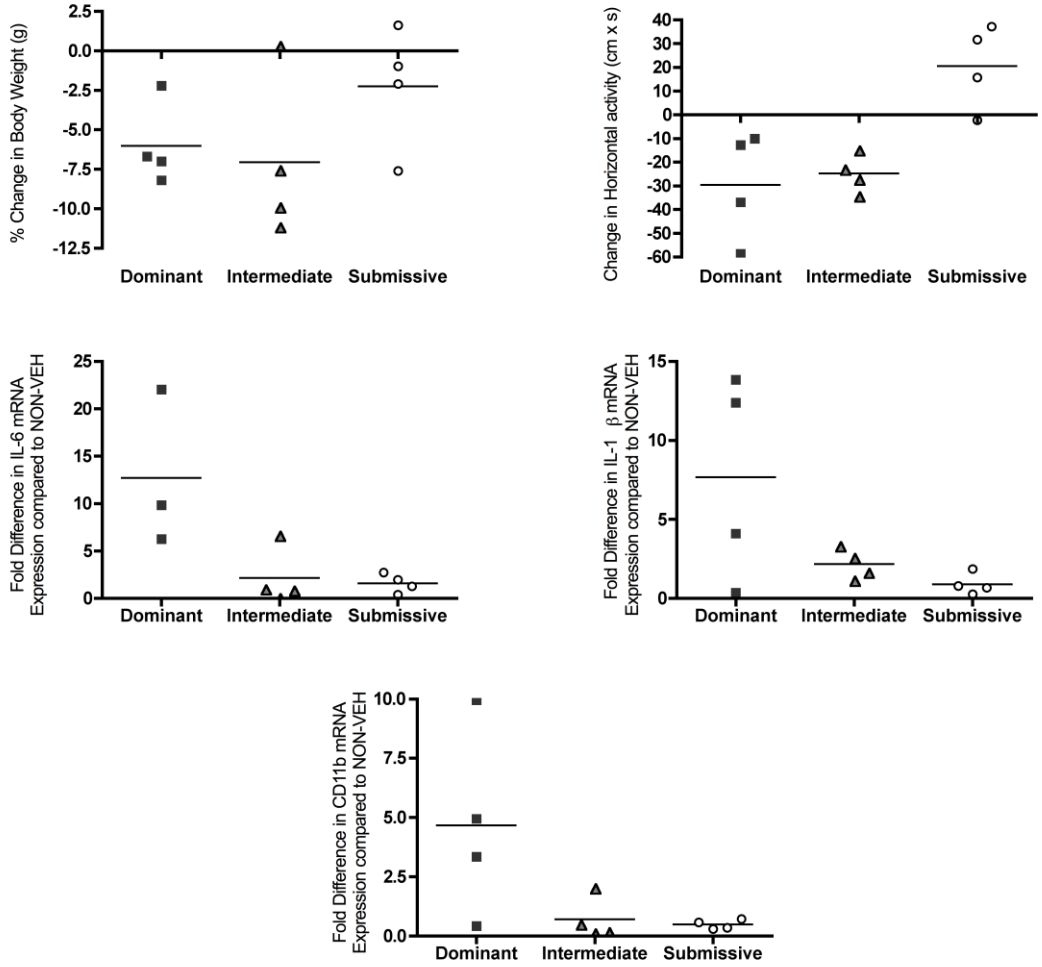
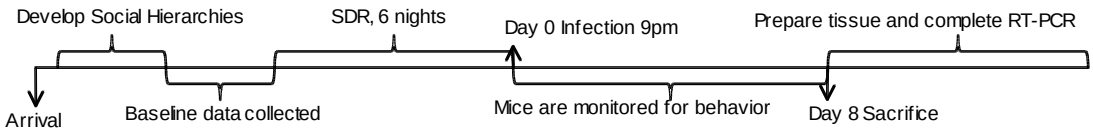


Figure 8. There are noticeable individual differences among cage mates. Here, although there are no statistically significant differences, we show that these individual differences may be due to social rank. The submissive animal seems to be behaviorally protected from the exacerbating effects of SDR while the dominant seems to be driving the inflamatory effects of social stress.

Discussion

The individual differences in reactivity and immune response among cage mates in a pattern that is seemingly related to social rank is supportive of the theory that social rank is a mediator of the priming effects of SDR. Contrary to Avitsur, Stark, and Sheridan (2001), however, it seems that in our experiments we are seeing that the dominant mouse is the one most affected in CNS inflammation by SDR and that the subordinate mouse is behaviorally protected from SDR induced exacerbation of acute TMEV. We could perhaps attribute these contrary findings to a difference in aggressiveness of both the experimental and the dominant intruder mice. The aggressive differences could be attributed to a strain difference, Avitsur, Stark, and Sheridan (2001) used C57BL/6 mice, quite an aggressive strain, while we used BALB/cJ mice, a strain chosen for their TMEV susceptibility but one that is known for their high anxiety levels. Another possible reason for observed differences in aggressiveness could be age; while Avitsur, Stark, and Sheridan (2001) used mice aged 2-4 months old who are well into sexual maturity, while we use mice of adolescent age (about 3 weeks), due to time restrictions of viable TMEV infection. An additional difference between Avitsur, Stark, and Sheridan's 2001 findings and ours is that they only looked at glucocorticoid resistance, while we have many behavioral and inflamatory dependent variable, not necessarily connected directly to glucocorticoid resistance. We see this trend in percent body weight loss, horizontal activity, and in mRNA expression of IL-6, IL-1B, and CD11b. These findings support the need for further investigation in the mediating role of social rank on SDR induced exacerbation of acute TMEV infection.



Experiment 3b Procedure

We further examined the modulating effects of social rank on SDR exacerbation of acute TMEV infection by employing a two group design (SDR vs Non SDR) with 12 subjects in the SDR condition and 10 subjects in the Non SDR condition. Upon arrival mice were group housed two to a cage and given five days without experimental manipulation to establish a social hierarchy. During this time, baseline data were collected for body weight, sucrose preference, food consumption, activity, mechanical sensitivity, hind limb impairment, and clinical score. Behavioral measures were collected throughout the experiment. After a five day period mice were tested for social rank using the food competition test, sexual preference test, the resident intruder test, and behavior during SDR for mice in the SDR condition. Instead of a counterbalanced design, the order of the social rank tests were chosen to place the most stressful, and therefore most obtrusive test, last in order to minimize carry over. After three days of testing the mice in the SDR condition underwent SDR for six nights starting at 5pm. Two hours after the last session of SDR all mice were inoculated with TMEV. Mice were behaviorally monitored for eight days post infection and then sacrificed. After the end of the experiment the first session of SDR was video scored for social rank using the Avitsur, Stark, and Sheridan (2001) method.

Results

The results of the social rank tests were analyzed utilizing linear hierarchical regression with stress in the first block, the social rank continuous variable as the second and/or third blocks, and the interactions of the two social rank variables as the fourth and/or fifth blocks to determine how well they predicted individual behavioral and inflamatory differences in responses to stress and infection. Linear hierarchical regression was chosen to allow analysis of both categorical and continuous independent variables. Please note that the food competition independent variable was not used due to insufficient fights over food and therefore inconclusive social rank data. As shown in Table 1, there were few significant interactions between SDR and social rank, contrary to predictions. Resident intruder aggressive ratio did significantly predict some variables, including decreases in body weight and movement and increases in rest time although these results are not interpreted as clinically significant predictors.

One-way ANOVAs were utilized to test the effects of chronic SDR on sickness behaviors after infection with TMEV. Significant effects of stress were revealed for mechanical sensitivity day 1 post infection change from baseline, $F(1,20)=13.203$, $p<0.05$, hind limb impairment day 4 change from baseline, $F(1,20)=7.722$, $p<0.05$, and day 7 change from baseline, $F(1,20)=4.946$, $p<0.05$, clinical score day 4 change from baseline, $F(1,20)=4.687$, $p<0.05$ (see Figure 9). Additionally stress significantly decreased activity and significant main effects were found for movement day 4 change from day -7 baseline $F(1,20)=12.923$, $p<0.05$, rest time day 4 change from baseline,

$F(1,20)=4.577$, $p<0.05$, and horizontal activity day 4 change from baseline, $F(1,20)=19.694$, $p<0.05$, and day 7 change from baseline, $F(1,20)=19.837$, $p<0.05$ (see Figure 10). No other behavioral measures differed significantly across SDR groups, all $p>0.05$.

As noted, we observed some individual differences in the data that could not be explained by stress or social rank. To examine whether baseline differences in anxiety might account for individual differences in the impact of SDR on infection, we analyzed baseline center time activity as a proxy variable for anxiety (cf. Prut & Belzung, 2003). An ANCOVA, using baseline center duration as a covariate, on the variables that were found to differ significantly across stress to determine if anxiety explained some of the individual variance seen in the data. These *post hoc* analyses revealed that when center duration was entered as a covariate it explained so much variance that SDR no longer significantly affected acute TMEV infection, suggesting that individual differences in baseline anxiety mediate the effects of SDR on infection. These variables included change in hind limb impairment at day 7, change in encephalitis at day 4, change in rest time at day 4, and change in horizontal activity at day 7.

Table 1

Linear Hierarchical Regression using Stress and Social Rank as predictors

	Regression Coefficients (B)				
Outcome Variable	SDR	RIAR	SPR	RIAR X Stress	SPR X Stress
Fur Score pre SDR2	-0.064	-0.616	0.001	-2.475	0.443
Fur Score pre SDR3	*0.227	0.043	-0.491	0.714	0.143
Fur Score Pre SDR4	-0.050	-0.004	-0.095	0.073	0.333
Fur Score Pre SDR5	-0.086	-0.157	0.130	0.989	-0.428
Fur Score Pre SDR 6	0.032	0.568	-0.297	-0.422	0.983
Von Frey Day 1 change	*0.807	-1.045	0.643	-1.475	0.532
Von Frey Day 4 change	0.328	-1.408	1.132	0.446	-2.832
Von Frey Day 7 change	0.054	-1.040	* 1.191	-0.561	-1.334
HLI Day 1 change	0.679	-1.766	0.871	0.391	1.309
HLI Day 4 change	* 1.446	-2.635	-1.709	0.171	0.151
HLI Day 7 change	* 1.258	-3.998	0.206	0.466	-2.301
Enc Day 1 change	0.104	0.018	* 0.617	-1.120	0.803
Enc Day 4 change	* 0.268	-0.618	0.655	0.076	0.224
Enc Day 7 change	0.068	0.012	0.122	-0.863	0.438
BBB Day 1 change	0.317	-2.672	-0.639	* 10.385	1.910
BBB Day 4 change	0.467	-0.293	0.936	6.841	-1.379
BBB Day 7 change	0.267	0.788	0.296	5.126	7.265
BW Day 1 change, from day -6	-0.361	-3.390	-1.207	0.698	-0.204
BW Day 2 change, from day -6	-0.264	* -4.609	-1.212	-1.708	-0.055
BW Day 3 change, from day -6	0.113	*-5.048	-2.661	0.291	0.515
BW Day 4 change, from day -6	0.134	* -4.514	-2.053	0.597	1.504
BW Day 5 change, from day -6	-0.098	-3.825	-1.967	0.938	0.601
BW Day 6 change, from day -6	-0.186	-3.636	-2.210	1.117	1.312
BW Day 7 change, from day -6	-0.014	-3.320	-1.346	0.568	0.673
Center Duration day 1 change	1.499	-2.192	-0.790	3.557	8.087
Center Duration day 4 change	-1.866	-6.249	-2.326	5.918	11.349
Center Duration day 7 change	-0.006	-4.407	-1.298	13.879	14.241
Center Entries day 1 change	0.353	-0.769	-0.340	0.904	2.266
Center Entries Day 4 change	-0.619	-1.217	0.346	-1.398	4.323
Center Entries Day 7 change	-0.037	-0.800	0.284	1.668	3.164
Movement Day 1 change	0.709	-5.379	-2.335	6.109	0.340
Movement Day 4 change	* -1.979	* -9.468	1.641	1.351	1.423
Movement Day 7 change	-0.135	-0.953	2.134	6.524	2.980
Rest time Day 1 change	-1.858	5.722	2.244	-8.736	-2.108
Rest time Day 4 change	* 1.868	* 9.785	-0.585	-0.780	-5.656
Rest time Day 7 change	-0.132	2.002	-1.059	-10.075	-7.658
Vertical Activty Day 1 change	3.310	-27.515	-17.808	15.833	-5.657
Vertical Activity Day 4 change	-2.537	-3.016	0.563	-21.734	0.040
Vertical Activity Day 7 change	-2.872	-9.338	-12.340	-23.391	-30.556
Horizontal Activity Day 1 change	11.256	-70.072	-55.257	10.676	-11.291
Horizontal Activity Day 4 change	-18.657	-34.387	10.488	-56.959	-24.710
Horizontal Activity Day 7 change	-12.972	1.266	16.704	-105.620	-42.735

Resident intruder aggressive ratio (RIAR)= Duration experimental animal was aggressive toward other animals (both cage mate and young intruder)/ total social activity duration

Sexual preference ratio (SPR) = Female Duration spent in experimental mouse arm/Total female duration in arms

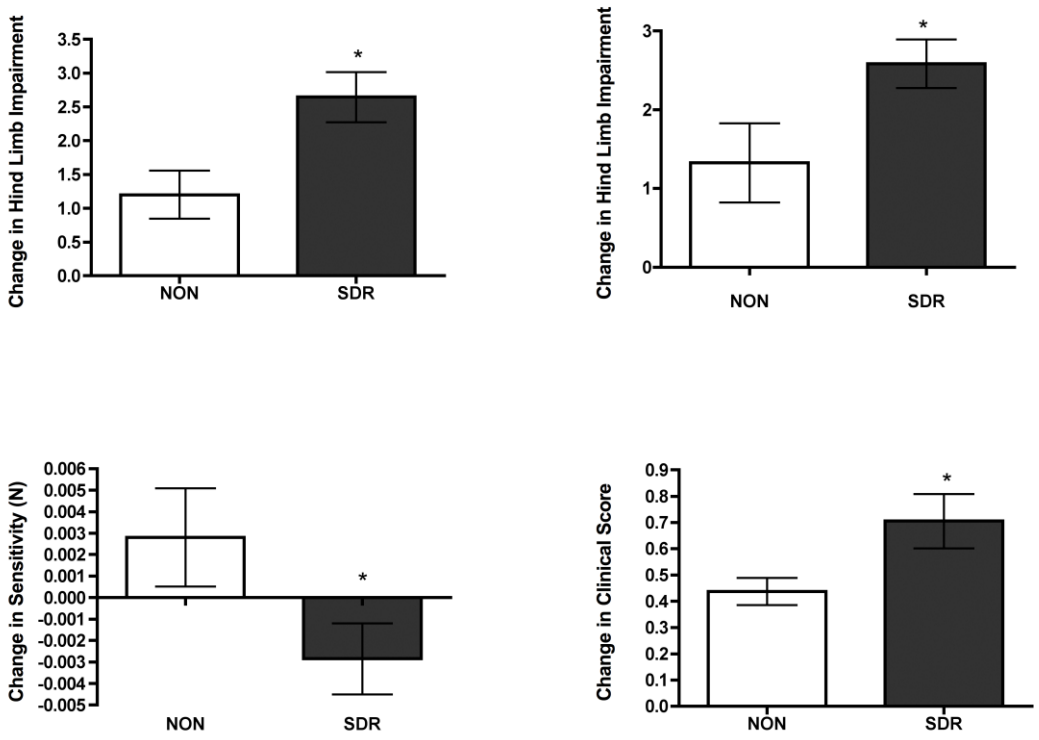


Figure 9. Blind clinical scoring was employed to quantify illness symptoms. Clinical score is a score of clinical illness while hind limb impairment was employed as a measure of motor impairment. Mechanical Sensitivity is a test of hyperalgisia. SDR significantly exacerbated Hind Limb impairment on day four and seven post infection, clinical score at day four, and mechanical sensitivity at day one post infection, all $p>.05$. Asterisks indicate significant post hoc differences between groups. Abbreviations for the experimental treatments are as follows: Non-SDR (NON) and social disruption (SDR).

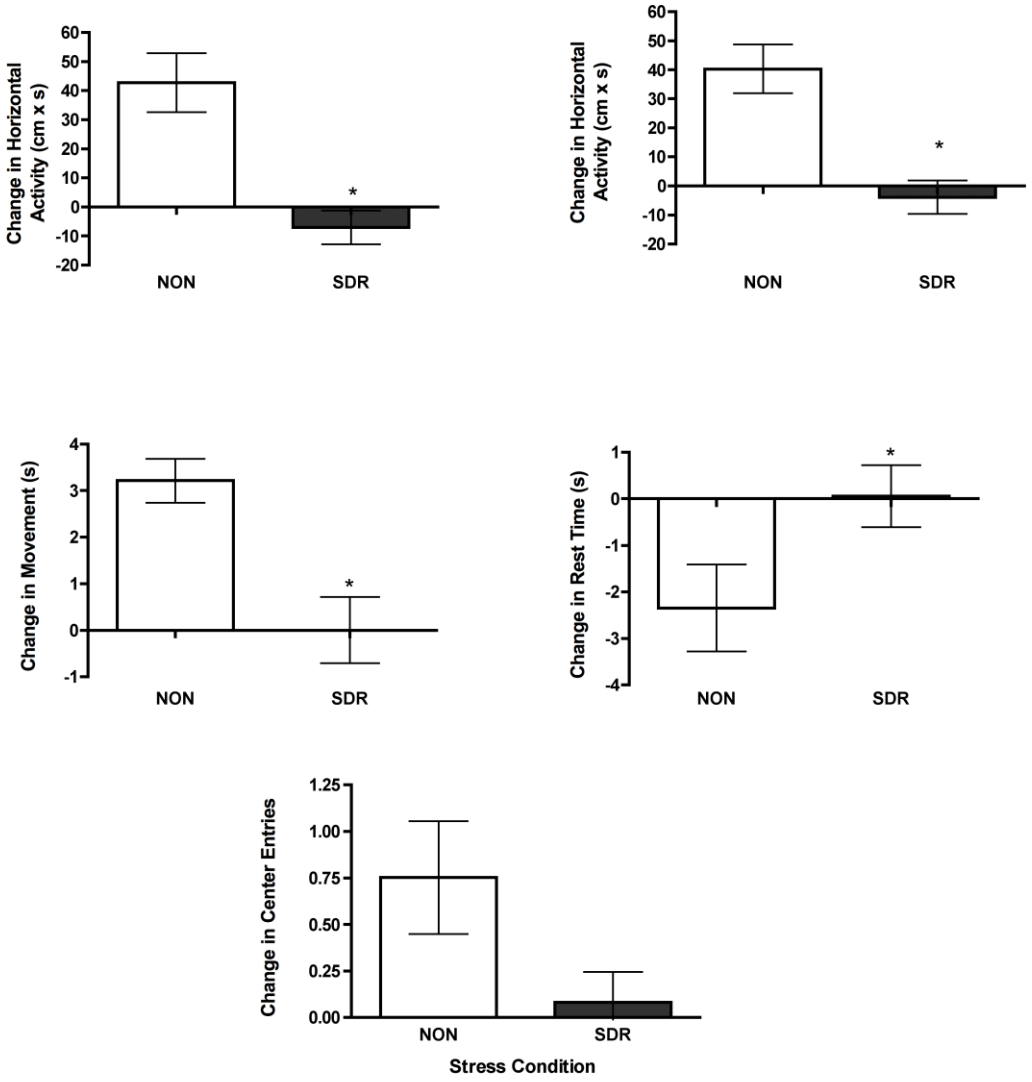


Figure 10. Horizontal activity, movement, and rest time were used as a measure of lethargy. Horizontal activity was significantly depressed at day four and seven post infection, movement was significantly depressed at day four post infection, while rest time was significantly increased at day four post infection all $p>0.05$. Center entries were used as a measure of anxiety, while not less than $p=0.05$, this measure did approach significance with $p=0.055$. Asterisks indicate significant post hoc differences between groups. Abbreviations for the experimental treatments are as follows: Non-stressed (NON), social disruption (SDR).

Discussion

While resident intruder aggressive ratio did significantly predict some variables, including decreases in body weight and movement and increases in rest time, these effects may be attributable to a relationship between body weight and general aggressiveness and are therefore not as important for understanding the relationship between SDR and acute TMEV infection. There were no clinically relevant results for other social rank variables and we therefore must conclude that, in our paradigm, social rank is not a significant moderator of SDR induced exacerbation of disease.

Although we did not replicate the results seen in Avitsur, Stark, and Sheridan 2001, our results are still interesting and important. The differences between our results and those of Avitsur, Stark, and Sheridan 2001 may be attributable to differences in the age and strain of our mice as well as to consequent differences in level of aggression. Avitsur, Stark, and Sheridan use adult mice aged two-four months in their research while we, due to TMEV susceptibility, must use adolescent mice aged about three weeks. Unlike adult mice that tend to be territorial and aggressive, adolescent mice have been observed actively cuddling each other and display far less aggressive tendencies than adult male mice. Our findings that social rank does not significantly mediate the relationship between SDR and acute TMEV infection may be due to the lower levels of overall aggressiveness observed in adolescent mice. Additionally, while we choose to use BALB/cJ mice due to their susceptibility to the acute phase of TMEV, this strain is known to be high in anxiety and therefore more timid than the quite aggressive C57BL/6 strain of mouse used by Avitsur, Stark, and Sheridan's (2001). Further support for this

hypothesis is provided by a comparison of levels of SDR-induced wounding across laboratories. Overall, we see far less wounding in our adolescent mice that Avitsur, Stark, and Sheridan (2001) observe in their aggressive adult mice (they observed mice with "deep lesions of the skin, genital area, or tail" while we observed *at most* one or two small tail bites).

Although our initial hypothesis was not supported, the results from Experiment 3 have some important implications for this line of research. First, we found that SDR significantly exacerbates acute TMEV infection even when mice are housed 2 to a cage instead of 3 to a cage. This is an important finding because it allows us to group house animals 2 to a cage in future studies which can both decrease costs and increase statistical power for group measured variables, such as food consumption and sucrose preference. Additionally, we found that center duration baseline (as a proxy variable for baseline anxiety) used as a covariate significantly accounted for a portion of the variance seen in the data above and beyond the variance accounted for by SDR. These results suggest that the individual variability observed in our research on the relationship between SDR and acute TMEV infection could be attributed to baseline levels of anxiety. It should be noted that starting with this experiment more careful attention/selection was used for the intruders and this may be why we see more robust behavioral effects from SDR in this study than in Experiment 1 or 2.

GENERAL DISCUSSION AND CONCLUSIONS

The present study was designed to test the hypothesis that repeated exposure to SDR prior to infection sensitizes the inflamatory response to TMEV. Previously, our laboratory established that prior exposure to SDR exacerbated acute TMEV infection by up-regulating central inflammation, inducing GCR, and aggravating behavioral measures of sickness (Johnson et al., 2004). Furthermore, previous research found that prior exposure to SDR exacerbated the acute and chronic phase of TMEV and, importantly, that disease course and circulating levels of IL-6 in the acute phase predicted chronic phase onset and development (Johnson et al., 2006). Subsequent research revealed that IL-6 is necessary for SDR induced exacerbation of TMEV infection, specifically that intracranial administration of IL-6 AbTx during the stress exposure period prevented exacerbation (Meagher et al., 2007). The objective of this thesis was to determine whether the adverse behavioral and neuroimmune effects of SDR on acute TMEV infection are mediated by the sensitization central inflamatory mechanisms.

To better understand the role of SDR-induced IL-6 and its mechanism of action, Experiment 1 examined SDR induced increases in central inflammation following both one session and six sessions of SDR. Based on our sensitization hypothesis and previous work (Meagher et al., 2007), we predicted that repeated exposure to SDR would sensitize the IL-6 response following 6 SDR sessions, but not after the first SDR session. Supporting this view, IL-6 mRNA expression was up-regulated immediately following and 12 hours after six sessions of SDR. Additionally, the effect of SDR on IL-1β expression habituated over time: after one session there was a robust response but by the

sixth session there was no response. This could indicate that IL-1β is involved in the inflamatory processes of SDR, but may be more of a trigger for further inflammation rather than the sole mechanism (Maier &Watkins, 1998).

Experiment 2 examined whether the negative effects of SDR on TMEV infection are mediated by stress-induced increases in central IL-6, which sensitizes virus-induced CNS inflammation. We predicted that SDR would increase virus-induced cytokine expression and that intracranial infusion of a neutralizing antibody to IL-6 during the stress exposure period would prevent this effect. Our hypothesis was supported, demonstrating that SDR-induced increases in central IL-6 during the stress exposure are necessary to the subsequent sensitization of virus-induced IL-1β and IL-6 cytokine expression. Moreover, we found that SDR increased the expression of a marker for microglial activation, CD11b, These findings provide further support that IL-6 up-regulation provides a possible mechanism mediating SDR related disease exacerbation.

While SDR did cause increased gene expression indicative of increased inflammation within the CNS, this did not translate into behavioral effects: SDR did not result in significant behavioral indication of sickness. There are several potential explanations for this negative finding. One possibility is that intensity of our SDR stress effect was attenuated because we did not verify the aggressiveness of the dominant male intruders used in this study. Normally, the intruder mice are rigorously preselected prior to SDR to ensure the reliability of our independent variable. However, due to methodological drift in the laboratory, this was omitted. During Experiment 2 there had been a drift from previous studies in the dominant intruder selection process: only one

round of dominance testing was completed and aggressiveness of intruders was not thoroughly confirmed. Thus, while the level of SDR aggression may have been sufficient to induce changes in CNS inflammation, they may have not been high enough to induce significant effects on our behavioral tests. This data, showing that SDR induces increased CNS inflammation but not measureable sickness behaviors, suggests that CNS inflamatory variables are more sensitive to social stressors than behavioral variables. Another possibility is that methodological drift and variability in the measures of sickness behavior may have attenuated these effects. Unlike our prior studies where the dependent measures were collected by two graduate students and two undergraduate honors fellows students, the data for this experiment were collected by a large number of less experienced undergraduate research assistants. Finally, it is possible that the combination of noise in both the independent and dependent variables contributed.

Importantly, when we returned to the more rigorous intruder selection criteria and provided careful supervision of the dependent measures of sickness behavior, a robust effect of SDR on sickness behavior exacerbation was observed in Experiment 3. This is consistent with previous studies where SDR exacerbated behavioral measures of sickness and motor impairment (Johnson et al., 2004, 2006; Meagher et al., 2007). Thus, it is likely that differences observed in the strength of SDR to induce disease exacerbation between Experiment 2 and Experiment 3 may be due to these changes in procedure. This drift in procedure could be the reason that the behavioral findings in Experiment 3 were more robust (and matching previous studies) than in Experiment 2.

Previous research has shown that social factors, such as rank in the social hierarchy, modulate the effects of social stressors (Audet & Anisman, 2009; Avitsur et al., 2007; Avitsur, Stark, & Sheridan, 2001; Bento, Dalrymple-Alford, & Brain, 1980; Fauman, 1987; Ferrari et al., 1997; Haemishch, Voss, & Gartner, 1993; Merlot et al., 2004). Specific to SDR paradigm, Avitsur, Stark, and Sheridan (2001) found that the most submissive mouse in a cage was the only one to develop GCR after SDR. Additionally, the most submissive mice had the highest incidence of wounding. They concluded that social rank modulates wounding and that wounding was, in turn, an important mediator of the physiological effects SDR. Later research supported this hypothesis with the finding that dominant mice were more likely to show an active response to defeat and received less bite wounds than submissive mice that showed a more robust glucocorticoids resistance and splenomegaly response to SDR (Avitsur et al., 2007). Avitsur and colleagues (2007) concluded that, because all subordinates received a number of bite wounds while the dominants received mostly superficial wounds, that the effect of social rank was probably based on the likelihood of being injured.

As a consequence of these findings, we explored social rank as a possible modulating factor that might account for unexplained variance in the effect of SDR on acute TMEV infection. We also measured wounding in our model. We found that social rank, as defined by aggressive behavior in the resident intruder test and sexual preference is not a significant modulator of SDR-related exacerbation of TMEV. Additionally, we observed a lower incidence and severity of wounding than levels

reported in previous research (Avitsur et al., 2007; Avitsur, Stark, and Sheridan, 2001). The differences between current findings and past research are probably due to age and strain differences.

Due to constraints placed by TMEV infection time and susceptibility, we use adolescent BALB/cJ mice while past research on SDR has used more aggressive adult C57BL/6 mice (Avitsur, Stark, and Sheridan, 2001; Avitsur et al., 2007, Quan et al., 2001). At the time of SDR, our mice were aged at about three weeks old, where as Sheridan's mice ranged between two and four months of age. These differences in age and strain of mouse resulted in less wounding overall and, if we base our theory on Avitsur, Stark, and Sheridan's (2001) conclusions, no modulating effect of social rank. At most we observed one or two superficial wounds while Avitsur, Stark, and Sheridan (2001) reported high levels of wounding, including multiple and severe bites to the tail and torso. It is important to note that, while we did not see the same quantity or severity of wounding, we did see SDR-induced exacerbation of acute TMEV infection. This would lead us to conclude that SDR is a psychological stressor in adolescent mice that is not reliant upon wounding.

Additionally, we provided evidence that baseline anxiety was a significant mediator of SDR-induced exacerbation of acute TMEV infection. Previous research noted that submissive mice were less likely to retaliate against dominant intruders with aggressive offensive behavior (Avitsur et al., 2007). Avitsur and colleagues (2007) concluded that this led to more wounding and therefore more effect of SDR but, with our current findings, an alternate hypothesis can be elucidated. Perhaps animals labeled

submissive are actually higher in baseline anxiety and instead of failing so show aggressive retaliation behavior are instead exhibiting anxiety induced avoidance behavior. Thus, they may have sustained greater wounding due to anxiety inhibiting their aggressive behavior. This is an empirical question that should be explored further in the future by testing baseline anxiety and comparing it to aggressive retaliation behavior during SDR.

Implications

SDR, a psychological social stressor, increased CNS inflammation and exacerbated acute TMEV infection. Importantly, IL-6 AbTx prevented SDR induced CNS inflamatory exacerbation of TMEV infection. Human research indicates that stressful life events deregulate inflammation in the body and cause permanent neuronal changes (McEwen, 2007). Additionally, stressful life events have been shown to exacerbated degenerative diseases, such as MS (Mohr et al., 2004). Knowing this, along with our findings, we can hypothesize that anti-inflamatory interventions or alternative treatments shown to decrease the expression of pro-inflamatory cytokines such as exercise, directed meditation, or omega-3 fatty acids could be used to reduce the risk or severity of degenerative disease in stressed individuals at risk of CNS inflamatory disease (Carlson et al., 2003; Gielen et al., 2003; Kenis & Maes, 2002; Simopoulos, 2002). We found that IL-6 is necessary for SDR induced exacerbation of acute TMEV infection, while other research has shown that IL-1β is necessary for SDR induced GCR, so a treatment specifically targeting these pro-inflamatory cytokines would be ideal (Engler et al., 2008).

Additionally, our experiments revealed that baseline anxiety mediated the effect of SDR on acute TMEV infection: the higher baseline anxiety, the more observed exacerbation. This finding suggests that patients with higher levels of emotionality or anxiety should use more caution to avoid or attenuate the effects of stressful life events. Some possible preventative measures include anti-anxiety medication or alternative treatments such as directed behavioral and schema modification, omega-3 fatty acids, meditation/mindfulness relaxation training, and daily exercise (Carlson, et al., 2003; Gielen et al., 2003; Kenis & Maes, 2002; Simopoulos, 2002).

Future Directions

Future studies of SDR related exacerbation of disease should take the findings of this thesis into consideration for direction and caution. First, the differences in behavioral exacerbation observed between Experiments 2 and 3 emphasize the importance of maintaining consistent behavioral protocols for ensuring the reliability of the independent variable (SDR) and the testing procedures across studies. Experiment 3 showed that SDR is an effective stressor even with only two home cage mice, so in future studies we should consider group housing mice two to a cage. This would allow us to test more groups with the same number of mice and would increase the power of group measures, such as sucrose preference and food consumption.

Additionally, Experiment 3 findings revealed that baseline anxiety is an important mediating factor of the effects of SDR. If we measure baseline anxiety and use it as a covariate in future studies, we could increase our statistical power. A simple way to measure anxiety, in addition to baseline center activity, is to count the number of

defecations in a novel environment (Crawley et al., 1997). We could easily implement this measure without disrupting current protocols by counting after the first activity monitoring habituation. Additionally, it would be interesting to measure baseline stress using the levels of corticosterone in these defecations.

Peripheral tissue and sera were collected in all current experiments, but due to time constraints and specified hypotheses, was not processed and tested. Because Merlot and colleagues (2003) have previously observed increased circulating levels of IL-6 after a single session of SDR, we expect that we would observe the same phenomena in sera after a single session of SDR. Additionally, previous research has shown SDR induced increases in circulating IL-6 levels (Johnson et al., 2006). Therefore, we should test for inflamatory marker expression in the spleens of animals from Experiments 1 and 2 in order to confirm SDR induced increases in peripheral inflammation. In order to complete the story of SDR induced CNS inflammation and TMEV exacerbation; we should also explore anti-inflamatory markers, such as IL-10.

Finally, Experiment 2 showed that IL-6 is necessary for SDR induced exacerbation of acute TMEV infection indicating that IL-6 up-regulation is a possible mechanism for exacerbation. A study showing that IL-6 is both necessary and sufficient for exacerbation of acute TMEV infection would provide further support of this theory. Therefore, a future study should test the hypothesis that central IL-6 up-regulation is sufficient for TMEV exacerbation.

In summary, findings from the present studies indicate that SDR increases central inflammation and that CNS inflammation may be more a more sensitive measure of

stress than behavioral measures. We found that prior exposure to SDR exacerbates acute TMEV infection and that a central infusion of IL-6 AbTx prevents exacerbation. Additionally, we found that, in our paradigm, social rank is not a significant mediator of SDR exacerbation of acute TMEV infection but that baseline anxiety is a significant mediator. We suggest preventative treatment for stress related disease exacerbation, such as specified anti-inflamatory or anti-anxiety medication in addition to alternative treatments such as exercise, mindfulness relaxation, or omega-3 fatty acids. Finally, we advise that the findings of this thesis be taken into consideration for future studies of SDR-induced inflammation and exacerbation of disease.

REFERENCES

Ackerman, K. D., Martino, M., Heyman, R., Moyna, N. M., & Rabin, B. S. (1998). *Psychosomatic Medicine, 60,* 484-491.

Ackerman, K.D., Heyman, R., Rabin, B.S., Anderson, B.P., Houck, P.R., Frank, E., & Baum, A. (2002). Stressful life events precede exacerbations of multiple sclerosis. *Psychosomatic Medicine, 64*, 916–920.

Audet, M. & Anisman, H. (2009). Neuroendocrine and neurochemical impact of aggressive social interactions in submissive and dominant mice: implications for stress-related disorders. *International Journal of Neuropsychopharmacology 10.1017*, 1-12.

Avitsur, R., Kinsey, S.G., Bidor, K., Bailey, M.T., Padgett, D.A., & Sheridan, J.F. (2007). Subordinate social status modulates the vulnerability to the immunological effects of social stress. *Psychoneuroendocrinolog,y 32*, 1097-1105.

Avitsur, R., Pollak, Y., & Yirmiya, R. (1997). Different receptor mechanisms mediate the effects of endotoxin and interleukin-1 on female sexual behavior. *Brain Research, 773*, 149-161.

Avitsur, R., Stark, J.L., & Sheridan, J.F., (2001). Social stress induces glucocorticoid resistance in subordinate animals. *Hormones and Behavior, 39*, 247–257.

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A. S., Dantzer, R., & Parmigiani, S., (2005). Social factors and individual vulnerability to chronic stress exposure. *Neuroscience and Biobehavioral Reviews, 29*, 67-81.

Basso, M. D., Fisher, L. C., Anderson, A. J., Jakeman, L. B., McTigue, D. M., & Popovich, P. G. (2006). Basso mouse scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. *Journal of Neurotrauma, 23 (5),* 635-659.

Benton, D., Dalrymple-Alford, J. C., & Brain, P. F. (1980). Comparisons of measures of dominance in the laboratory mouse. *Animal Behavior, 28,* 1274-1279.

Blandino P. J., Barnum, C., & Deak, T. (2006). The involvement of nerepinephrine and microglia in hypothalamic and splenic IL-1beta responses to stress. *Journal of Neuroimmunology, 173*, 87-95.

Bluthè, R., Michaund, B., Poli, V., & Dantzer, R. (2000). Role of IL-6 in cytokine induced sickness behavior: a study with IL-6 deficient mice. *Physiology & Behavior, 70,* 367-373.

Carlson, L. E., Speca, M., Patel, K. D., & Goody, E. (2003). Mindfulness-based stress reduction in relation to quality of life, mood, symptoms of stress, and immune parameters in breast and prostate cancer outpatients. *Psychosomatic Medicine, 65,* 571-581.

Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L, et al. (1997) Behavioral phenotypes of inbread mouse strains: implications and recommendations for molecular studies. *Psychopharmacology, 132,* 107-124.

Dantzer, R., (2001). Cytokine-induced sickness behavior: Mechanisms and implications. *Annals New York Academy of Sciences,* 222-234.

Dantzer, R. & Kelley, K.W., (2007). Twenty years of research on cytokine induced sickness behavior. *Brain Behavior and Immunology, 21*, 153–160.

Dantzer, R., Wollman, E., Vitkovic, L., & Yirmiya, R. (1999). Cytokine and depression: fortuitous or causative association? *Molecular Psychiatry, 4,* 328-332.

Dal Canto & Vanderlugt (2005). The role of astrocytes, oligodendrocytes, microglia and endothelial cells in TMEV infection. Eds., E. Lavi and S. Constantinescu *In Experimental Models of Multiple Sclerosis*, (pp. 617 – 628) Kulwer Academic Publishers, London.

Deak, T., Bordner, K. A., McElderry, N. K., Barnum, C. J., Blandino, P., Deak, M. M., & Tammariello, S. P. (2005). Stress-induced increases in hypothalamic IL-1: a systematic analysis of multiple stressor paradigms. *Brain Research Bulletin, 64,* 541-556.

Deak, T., Meriwether, J. L., Fleshner, M., Spencer, R. L., Abouhamze, A., Moldawer, L. L., Grahn, R. E., Watkins, L. R., & Maier, S. F. (1997). Evidence that brief stress may induce the acute phase response in rats. *The American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 273*, 1998-2004.

Elenkov, I., Iezzoni, D. G., Daly, A., Harris, A. G., & Chrousos, G.P. (2005). Cytokine dysregulation, inflammation, and well-being. *Neuroimmunomodulation, 12,* 255 269.

Engeland, C. G., Nielsen, D. V., Kavaliers, M., & Ossenkopp, K. (2001). Locomotor activity changes following lipopolysaccharide treatment in mice: a multivariate assessment of behavioral tolerance. *Physiology & Behavior, 72,* 481-491.

Engler, H., Bailey, M.T., Engler, A., Stiner-Jones, L.M., Quan, N., & Sheridan, J.F. (2008). Interleukin-1 receptor type 1-deficient mice fail to develop social stress associated gludocorticoid resistance in the spleen. *Psychoneuroendocrinology, 33*, 108-117.

Fauman, M.A. (1987). The relation of dominant and submissive behavior to the humoral immune response in BALB/c mice. *Biological Psychiatry, 22*, 776-779.

Ferrari, P. F., Palanza, P., Parmingiani, S., & Rodgers, R. J. (1997). Interindividual variability in swiss male mice: Relationship between social factors, aggression, and anxiety. *Physiology & Behavior, 63 (5),821-827.*

Fitchett, A. E., Barnard, C. J., Cassady, H. J. (2009). Corticosterone differences rather than social housing predict performance of T-maze alternation in male CD-1 mice. *Animal Welfare, 18,* 21-31.

Flint, J., Corley, R., DeFries, J. C., Fulker, D. W., Gray, J. A., Miller, S., & Collins, A. C. (1995). A Simple genetic basis for a complex psychological trait in laboratory mice. *Science, 269,* 1432-1435.

Frank, M.G., Baratta, M.V., Sprunger, D.B., Watkins, L.R., & Maier, S.F. (2007). Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro inflamatory cytokine responses. *Brain, Behavior and Immunity, 21*(1), 47-59.

Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., & Yirmiya, R. (2008). Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Molecular Psychiatry, 13,* 717-728.

Gielen, R. S., Adams, V., Mobius-Winkler, S., Link, A., Erbs, S., Yu, J., et al. 92003). Anti-inflamatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. *Journal of American College of Cardiology, 42,* 861 868.

Haemishch, A., Voss, T., & Gartner, K. (1994). Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiology & Behavior, 56 (5),* 1041-1048.

Hurst, J. L., (2009). Female recognition and assessment of males through scent. *Behavioural Brain Research, 200,* 295-303.

Johnson, J. D., O'Connor, K. A., Deak, T., Stark, M., Watkins, L. R., & Maier, S. F. (2002). Prior stressor exposure sensitizes LPS-induced cytokine production *Brain, Behavior, and Immunity, 16,* 461-476.

Johnson, R.R., Prentice, T.W., Bridegam, P., Young, C.R., Steelman, A.J., Welsh, T.H., Welsh, C.J.R.,& Meagher, M.W., 2006. Social stress alters the severity and onset of the chronic phase of Theiler's virus infection. *Journal of Neuroimmunology, 175*, 39–51.

Johnson, R.R., Storts, R., Welsh Jr., T.H., Welsh, C.J., & Meagher, M.W., 2004. Social stress alters the severity of acute Theiler's virus infection. *Journal of Neuroimmunology, 148*, 74–85.

Kenis, G., & Maes, M. (2002). Effects of antidepressants on the production of cytokines. *International Journal of Neuropsychopharmacology, 5,* 401-412.

Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behavior: mechanisms and implications. *TRENDS in Neurosciences, 25(3),* 155-159.

Kelley, K. W., Bluthè R., Dantzer, R., Zhou, J., Shen, W., Johnson, R., & Broussard, S. R. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17,* S112-S118.

Lindzey, G., Winston, H., Manosevitz, M. (1961). Social dominance in inbread mouse strains. *Nature, 191,* 474-476.

Lipton, H.L., 1975. Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. *Infection and Immununity, 11*, 1147–1155.

Locurto, C., Benoit, A., Crowley, C., & Miele, A. (2006). The structure of individual differences in batteries of rapid acquisition tasks in mice. *Journal of Comparitive Psychology, 120,* 378-388.

Malloy, T. E., Barcelos, S., Arruda, E., DeRosa, M., Fonseca, C. (2005). Individual differences and cross-situational consistency of dyadic social behavior. *Journal of Personality and Social Psychology, 89,* 643-654.

Mackintosh, J. H. (1981). Behaviour of the house mouse. *Symposia of the Zoological Society of London, 47,* 337-365.

Maes, M., Lin. A., Delmeire, L., Gastel, A. V., Kenis, G., Jongh, R. D., & Bosmans, E. (1998). Elevated serum Interleukine-6 (IL-6) and IL-6 receptor concentrations in posttramatic stress disorder following accidental man-made traumatic events. *Society of Biological Psychiatry, 45,* 833-839.

Maier, S. F. (2003). Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition. *Brain, Behavior, and Immunity, 17,* 69-85.

Maier, S.F., & Watkins, L.R., (1998). Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychological Reviews, 105*, 83–107.

Matta, S. G., Weatherbee, J., & Sharp, B. M. (1992). A central mechanism is involved in the secreation of ACTH in response to IL-6 in rats: comparison to and interaction with IL-1 beta. *Neuroendocrinology, 56,* 516-525.

McEwen, B. S., (2007) Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiology Reviews, 87,* 873-904.

Meagher, M.W., Johnson, R.R., Young, E.E., Vichaya, E.G., Lunt, S., Hardin, E.A., Connor, M.A., & Welsh, C.J. (2007). Interleukin-6 as a mechanism for the adverse effects of social stress on acute Theiler's virus infection. *Brain, Behavior and Immunity, 21(8)*, 1083-1095.

Meagher, M.W. & Welsh C.J.R., (2009). Social stress and inflammation in the exacerbation of multiple sclerosis: An animal model with implications for humans. In K. Kendall-Tackett *The Psychoneuroimmunology of Chronic Disease: Exploring the Links Between Inflammation, Stress, and Illness* (pp. 159 181) Location: American Psychological Association

Merlot, E., Moze, E., Dantzer, R., & Neveu, P.J., (2003). Importance of fighting in the immune effects of social defeat. *Physiology and Behavior, 80*, 351–357.

Merlot, E., Moze, E., Dantzer, R., & Neveu, P.J., (2004). Cytokine production by spleen cells after social defeat in mice: activation of T cells and reduced inhibition by glucocorticoids. *Stress, 7*, 55–61.

Mi, W., Prentice, T. W., Young, C. R., Johnson, R. R., Sieve, A. N., Meagher, M. W., & Welsh, C. J. R. (2006). Restraint stress decreases virus-induced pro inflamatory cytokine mRNA expression during acute Theiler's virus infection. *Journal of Neuroimmunology, 178,* 49-61.

Mohr, D. C., Hart, S. L., Julian, L., Cox, D., & Pelletier, D. (2004). Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. *BMJ, doj:10.1136/bmj.38041.724421.55,* 1-5.

Mohr, D.C. & Pelletier, D., (2006). A temporal framework for understanding the effects of stressful life events on inflammation in patients with multiple sclerosis. *Brain Behavior and Immunology, 20*, 27–36.

Monteyne, P., Bureau, J., & Brahic, M. (1997). The infection of mouse by Theiler's virus: from genetics to immunology. *Immunological Reviews, 159,* 163-176.

Mossman, C. A. & Drickamer, L. C. (1996). Odor preferences of female house mice (*mus domesticus)* in seminatural enclosures. *Journal of Comparative Psychology, 110 (2),* 131-138.

Nair, A., & Bonneau, R.H. (2006). Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *Journal of Neuroimmunology*, *171(*1-2), 72-85.

Nguyen, K.T., Deak, T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., & Maier, S.F., (1998). Exposure to acute stress induces brain interleukin-1beta protein in the rat. *Journal of Neuroscience, 18*, 2239–2246.

O'Connor, K. A., Johnson, J. D., Hansen, M. K., Wieseler Frank, J. L., Maksimovo, E., Watkins, L. R., & Maier, S. F. (2003). Peripheral and central proinflamatory cytokine response to a severe acute stressor. *Brain Research, 991,* 123-132.

Pasquali, R., Anconetane, B., Chattat, R., Biscotti, M., Spinucci, G., Casimirri, F., Vicentina, V., Carcello, A., & Labate, A. M. M. (1996). Hypothalamic-Pituitary Adrenal axis activity and its relationship to the autonomic nervous system in women with visceral and subcutaneous obesity: Effects of corticotrophin-relasing factor/arginine-vasopressin test and of stress. *Metabolism, 45(3),* 351-356.

Perez, H. Sellings, L., Grieder, T., Diaz, J. (2009). Social dominance rank influences wheel running behavior in mice. *Neuroscience Letters, 457,* 137-140.

Perlstein, R. S., Whitnall, M. H., Abrams, J. S., Mougey, J. S., Neta, R. (1993). Synergistic roles of interleukin-6, interleukin-1, and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide *in vivo. Endocrinology, 132,* 946-952.

Perry, H. V., Cunningham, C., & Holmes, C. (2007). Systemic infections and inflammation affect chronic neurodegeneration. *Nature Reviews, 7,* 161-167.

Perry, V. H., Newman, T. A., & Cunningham, C. (2003). The impact of systemic infection on the progression of neurodegenerative disease. *Nature Reviews, 4,* 103-112.

Pollmacher, T., Haack, M., Schuld, A., Reichenberg, A., & Yirmiya, R. (2001). Low levels of circulating inflamatory cytokines-Do they affect human brain functions? *Brain, Behavior, and Immunity, 16,* 525-532.

Prut, L., Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European Journal of Pharmacology, 463,* 3-33.

Pugh, R. C., Nguyen, K. T., Gonyea, J. L., Fleshner, M., Watkins, L. R., Maier, S. F., & Rudy, J. W. (1999), Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. *Behavioural Brain Research, 106,* 109-118.

Quan, N., Avitsur, R., Stark, J.L., He, L., Shah, M., Caligiuri, M., Padgett, D.A., Marucha, P.T., & Sheridan, J.F. (2001). Social stress increases the susceptibility to endotoxic shock. *Journal of Neuroimmunology, 115*, 36–45.

Sato, S., Reiner, S. L., Jensen, M. A., & Roos R. P. (1997). Central nervous system cytokine mRNA expression following Theiler's murine encephalomyelitis virus infection. *Journal of Neuroimmunology, 76,* 213-223.

Sieve, A.N., Steelman, A.J., Young, C.R., Storts, R., Welsh, T.H., Welsh, C.J., & Meagher, M.W., (2004). Chronic restraint stress during early Theiler's virus Infection exacerbates the subsequent demyelinating disease in SJL mice. *Journal of Neuroimmunology 155*, 103–118.

Sieve, A. N., Steelman, A., J., Young, C. R., Storts, R., Welsh, T. H., Welsh, J. R., & Meagher, M. W. (2006). Sex-dependent effects of chronic restraint stress during early Theiler's virus infection on the subsequent demyelinating disease in CBA mice. *Journal of Neuroimmunology, 177,* 46-62.

Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune disease. *Journal of American College of Nutrition, 21,* 495-505.

Sospedra, M. & Martin, R. (2005). Immunology of multiple sclerosis. *Annual Review of Immunology, 23,* 683-747.

Stark, J.L., Avitsur, R., Hunzeker, J., Padgett, D.A., & Sheridan, J.F. (2002). Interleukin-6 and the development of social disruption-induced glucocorticoids resistance. *Journal of Neuroimmunology, 124*, 9–15.

Steelman A. J., Dean, D.D.,Young, C.R., Smith R., Prentice, T.W., Meagher M.W., & Welsh, C.J.R. (2009). Restraint stress modulates virus specific adaptive immunity during acute Theiler's virus infection. *Brain, Behavior, and Immunity, 23*, 830-343.

Sugama, S., Fujita, M., Hashimoto, M., & Conti, B. (2007). Stress induced morphological microglial activation in the rodent brain: Involvement of interleukin-18. *Neuroscience 146(3)*, 1388-1399

Young, E. E., Sieve, A. N., Vichaya, G. V., Carcoba, L. M., Young, C. R., Ambrus, A., Storts, R., Welsh, C. J., Meagher, M. W. (2010). Chronic restraint stress during early Theiler's virus infection exacerbates the subsequent deemyelinating disease in SJL mice:II. CNS desease severity. *Journal of Neuroimmunology, 220,* 79-89.

Zhou, D., Kusnecov, A. W., Shurin, M. R., Depaoli, M., & Rabin, B. S. (1993). Exposure to physical and psychological stressors elevates plasma interleukine 6: relationsip to activation of hypothalamic-pituitary-adrenal axis. *Endocrinology, 133,* 2523-2530.

APPENDIX

TABLE 1. SCORES AND OPERATIONAL DEFINITIONS FOR THE BASSO MOUSE SCALE FOR LOCOMOTION (BMS)

Score	
0	No ankle movement
1	Slight ankle movement
2	Extensive ankle movement
3	Plantar placing of the paw with or without weight support -OR- Occasional, frequent or consistent dorsal stepping but no plantar stepping
4	Occasional plantar stepping
5	Frequent or consistent plantar stepping, no coordination -OR- Frequent or consistent plantar stepping, *some* coordination, paws *rotated* at initial contact and lift off (R/R)
6	Frequent or consistent plantar stepping, *some* coordination, paws *parallel* at initial contact (P/R, P/P) -OR- Frequent or consistent plantar stepping, *mostly* coordinated, paws *rotated* at initial contact and lift off (R/R)
7	Frequent or consistent plantar stepping, *mostly* coordinated, paws *parallel* at initial contact and *rotated* at lift off (P/R) -OR- Frequent or consistent plantar stepping, *mostly* coordinated, paws *parallel* at initial contact and lift off (P/P), and *severe* trunk instability
8	Frequent or consistent plantar stepping, *mostly* coordinated, paws *parallel* at initial contact and lift off (P/P), and *mild* trunk instability -OR- Frequent or consistent plantar stepping, *mostly* coordinated, paws *parallel* at initial contact and lift off (P/P), and *normal* trunk stability and tail *down or up & down*
9	Frequent or consistent plantar stepping, *mostly* coordinated, paws *parallel* at initial contact and lift off (P/P), and *normal* trunk stability and tail *always* up.

Slight: Moves less than half of the ankle joint excursion.
Extensive: Moves more than half of the ankle joint excursion.
Plantar placing: Paw is actively placed with both the thumb and the last toe of the paw touching the ground.
Weight support: (dorsal or plantar): The hindquarters must be elevated enough that the hindend near the base of the tail is raised off of the surface and the knees do not touch the ground during the step cycle.
Stepping: (dorsal or plantar): Weight support at lift off, forward limb advancement and re-establishment of weight support at initial contact.
Occasional: Stepping less than or equal to half of the time moving forward.
Frequent: Stepping more than half the time moving forward.
Consistent: Plantar stepping all of the time moving forward with less than 5 missed steps (due to medial placement at initial contact, butt down, knee down, skiing, scoliosis, spasms or dragging) or dorsal steps.
Coordination: For every forelimb step a hindlimb step is taken and the hindlimbs alternate during an assessable pass. For a pass to be assessable, a mouse must move at a consistent speed and a distance of at least 3 body lengths. Short or halting bouts are not assessable for coordination. At least 3 assessable passes must occur in order to evaluate coordination. If less than 3 passes occur then the mouse is scored as having no coordination.
Some coordination: Of all assessable passes (a minimum of 3), most of them are *not* coordinated.
Most coordination: Of all assessable passes (a minimum of 3), most of them *are* coordinated.
Paw position: *Digits* of the paw are parallel to the body (P), turned out away from the body (external rotation: E) or turned inward toward midline (internal rotation; I).
Severe trunk instability: Severe trunk instability occurs in two ways.
(1) The hindquarters show severe postural deficits such as extreme lean, pronounced waddle and/or near collapse of the hindquarters predominantly during the test.

or

(2) Five or more of any of the following *events* stop stepping of one or both hindlimbs
- Haunch hit: the side of hindquarters rapidly contacts the ground
- Spasms: sustained muscle contraction of the hindlimb which appears to immobilize the limb in a flexed or extended position
- Scoliosis: lateral deviation of the spinal column to appear "C" shaped instead of straight

Mild trunk instability: Less than 5 events listed above and some sway in the hindquarters. Mild trunk instability is scored when the pelvis and haunches predominantly dip, rock, or tilt from side-to-side (tilt). If the tail is up, the swaying of the pelvis and/or haunches produces side-to-side movements of the distal third of the tail which also indicates mild trunk instability (side tail).
Normal trunk stability: No lean or sway of the trunk, and the distal third of the tail is steady and unwavering during locomotion. No severe postural deficits or events and less than 5 instances of mild instability.

Table 2. Scores and operational definitions for the Basso Mouse Scale for locomotion. Taken from Basso et al., 2006.

VITA

Name: Mallory Ann Frazier

Education: University of Mary Washington, B.S. in Psychology, 2007
Texas A&M University, M.S. in Psychology, 2010

Address: Care of: Dr. Mary Meagher
Texas A&M University
Department of Psychology, MS 4235
College Station Texas, 77843-4535