Published in final edited form as:

J Neuroimmunol. 2015 November 15; 288: 56–68. doi:10.1016/j.jneuroim.2015.09.005.

Social disruption alters pain and cognition in an animal model of Multiple Sclerosis

H.R. Linsenbardt^{a,b}, J.L. Cook^a, E.E. Young^a, E.G. Vichaya^a, C.R. Young^c, N.M. Reusser^a, R. Storts^d, C.J. Welsh^{b,c}, and M.W. Meagher^{a,b,*}

^aDepartment of Psychology, Texas A&M University, College Station, Texas

^bTexas A&M Institute for Neuroscience, Texas A&M University, College Station, Texas

^cDepartment of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas

^dDepartment of Veterinary Pathobiology, Texas A&M University, College Station, Texas

Abstract

Although pain and cognitive deficits are widespread and debilitating symptoms of multiple sclerosis (MS), they remain poorly understood. Theiler's murine encephalomyelitis virus (TMEV) infection is an animal model of MS where disease course is exacerbated by prior stressors. Here chronic infection coupled with prior social stress increased pain behavior and impaired hippocampal-dependent memory consolidation during the demyelinating phase of disease in SJL mice. These results suggest that the TMEV model may be useful in investigating pain and cognitive impairments in MS. However, in contrast with prior Balb/cJ studies, stress failed to consistently alter behavioral and physiological indicators of disease course.

Keywords

Multiple sclerosis; Theiler's murine encephalomyelitis virus; Pain; Pavlovian conditioning; Cognition; Social stress

1. Introduction

Multiple Sclerosis (MS) is a chronic disease affecting the central nervous system (CNS) characterized by neuroinflammation, demyelination, and neurodegeneration (Compston and Coles, 2008). Although research has focused on motor and sensory symptoms, recent evidence indicates that over 50% of patients with MS suffer from persistent pain (Truini et al., 2012) and cognitive deficits (Pardini et al., 2014). About 30% of MS patients report pain as their worst symptom (Beard et al., 2003), which can impair social and occupational functioning (Ehde et al., 2006) as well as mood and well-being (Hadjimichael et al., 2007).

*Corresponding author Mary W. Meagher, Telephone: +1 979 845 2564, m-meagher@tamu.edu, Physical Address: Texas A&M University, Psychology, 4235 TAMU, College Station, Texas 77843-4235.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Similarly, cognitive impairments, such as memory deficits (Covey et al., 2011, Amato et al., 2001), are disruptive to daily living (Amato et al., 1995, Amato, Ponziani, 2001, Rao et al., 1991). In addition, periods of psychological stress have been associated with the development and exacerbation of symptoms of MS, including pain and cognitive impairments, which will be discussed in more detail below (Ackerman et al., 2002, Brown et al., 2005, Brown et al., 2006a, b, Li et al., 2004, Mohr et al., 2004, Sorenson et al., 2013). Animal models are needed to further our understanding of MS-related pain and cognitive impairments and their underlying mechanisms.

Theiler's murine encephalomyelitis virus (TMEV) infection is a commonly used animal model of MS (Mecha et al., 2013, Welsh et al., 2009). Intracerebral inoculation of TMEV in susceptible mouse strains induces a biphasic disease of the CNS. The acute phase of disease is characterized by neuroinflammation due to neuronal and glial infection (Njenga et al., 1997) and behavioral signs of encephalitis and polio-like symptoms (Campbell et al., 2001, Johnson et al., 2006, Johnson et al., 2004, Sieve et al., 2004, 2006, Vichaya et al., 2011). During the acute phase, susceptible strains of mice fail to produce an effective early immune response resulting in viral persistence and subsequent immune-mediated demyelination during the chronic disease phase (Lipton and Melvold, 1984, Oleszak et al., 2004, Rodriguez et al., 1983) that mimics the demyelinating effects of MS in humans. Symptoms in mice progress from minor disruptions in gait, impaired motor coordination, and reduced locomotor activity at the early stages of chronic phase demyelination to profound dysfunction at the later stages due to myelin and axon loss (McGavern et al., 1999, McGavern et al., 2000).

Our laboratory has previously shown that repeated exposure to social disruption stress (SDR) prior to TMEV infection exacerbated motor impairments and sickness behaviors during both the acute and chronic phases of TMEV infection in Balb/cJ mice (Johnson, Prentice, 2006, Johnson, Storts, 2004, Meagher et al., 2007, Vichaya, Young, 2011, Young et al., 2013). These behavioral impairments are mediated by a stress-induced increase in the pro-inflammatory cytokine interleukin-6 (IL-6) that disrupts the initial immune response and increases the CNS viral persistence and neuroinflammation (Johnson et al., 2006, Meagher et al., 2007, Vichaya et al., 2011, Young et al., 2013). The present study examined whether the adverse effects of SDR prior to TMEV infection previously observed in Balb/cJ mice generalize to highly susceptible SJL mice. In addition to typical measures of disease progression (motor and sickness behaviors, antibodies to virus, spinal cord and brain lesions), we included measures of pain behavior and cognitive impairment to determine if this model system would be useful for studying these symptoms.

To measure the affective dimension of pain, we used a Pavlovian fear conditioning test. Prior research assessing pain sensitivity in the TMEV model relied on nociceptive reflex measures (Lynch et al., 2008a, Lynch et al., 2008b). While these measures provide information regarding spinal nociceptive processing (Gregory et al., 2013, Vierck et al., 2008), they do not reflect the affective experience of pain mediated by the brain (King et al., 2009, King et al., 1996, Meagher et al., 2001, Morgan et al., 1994, Qu et al., 2011, Vierck et al., 2008). In response to these concerns, researchers use conditioning tasks that assess the aversive experience of pain, similar to self-report measures in humans (Johansen et al.,

2001, King et al., 2006, King et al., 2009, LaBuda et al., 2001, LaBuda and Fuchs, 2000, Meagher et al., 2001, Qu et al., 2011). For example, contextual fear conditioning has been used to measure pain-related affective processing in rats (King et al., 2006, McLemore et al., 1999, Meagher et al., 2001). The present study used this approach to determine whether the affective experience of pain is enhanced by prior SDR during the chronic phase of disease. If the aversive experience of pain is increased in TMEV infected mice, they should perceive a mild footshock (unconditioned stimulus) as more aversive than the non-infected mice, which should enhance the acquisition of conditioned fear to the test chamber. This should lead to increased fear behavior when the animal is tested 24 hours after conditioning by measuring the level of conditioned freezing (conditioned response) when the mouse is re-exposed to the test chamber (conditioned stimulus; King et al., 1996, Meagher et al., 2001).

To measure the effects of stress on TMEV infection-induced impairments in cognition, we used the novel object recognition test during the chronic phase of disease. Prior studies have reported impaired cognitive function on a hippocampal-dependent place learning task during acute TMEV infection (Buenz et al., 2006, Howe et al., 2012a, Howe et al., 2012b) and the extent of cognitive impairment was significantly correlated with the amount of hippocampal damage in area CA1. However, no one has examined the effects of TMEV infection and prior SDR on cognitive functions during the MS-like chronic phase. The novel object recognition task, which exploits the natural tendency of mice to explore a novel object more than a familiar object, is advantageous because animals in the early stages of demyelination can readily perform the task. If hippocampal memory is impaired in infected mice, they will fail to remember the familiar object, indicated by equivalent levels of exploration of both the familiar and novel objects.

2. Materials and methods

2.1 Animals

Eighteen male SJL mice (22-25-days-old; Jackson Labs, Bar Harbor, ME) were maintained on a 12-h light/dark cycle (lights off at 17:00 h) with continuous white noise (64 dB) and ad libitum access to food and water. Animals were individually housed until they recovered from cannulation surgery, at which time they were counterbalanced and housed two per cage. Intruder mice were retired SJL breeders (10 mo old; Jackson Labs, Bar Harbor, ME). All animal care protocols were in accordance with the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

2.2 Experimental design

A mixed between- and within-subject design was used to examine the effects of social disruption stress (SDR) administered one week prior to infection on the acute and chronic phases of TMEV infection in SJL mice (Fig. 1). Subjects were randomly assigned either to SDR or remained undisturbed in their home cage. In an earlier study we did not observe an effect of SDR alone on a measure of motor behavior (Johnson et al., 2004). However, without an uninfected SDR group, we cannot definitively conclude that the changes in behavior observed during the chronic phase are attributable to infection and its interaction with stress. This resulted in assignment to one of three groups: 1) non-stressed and

uninfected (Non-SDR uninfected), 2) non-stressed and infected (Non-SDR infected), and 3) stressed and infected (SDR infected). Two hours after their final session of SDR subjects were infected. Acute phase measures were assessed at baseline through day 28 post infection (pi) to assess sickness behavior, clinical scores, hind limb impairment, and open field activity. To evaluate the demyelinating chronic phase of TMEV in SJL mice, clinical scores of ataxia and paresis, open field activity, footprint, rotarod, mechanical sensitivity, fear conditioning, novel object recognition, and immunological and histological analyses were recorded (see below for descriptions) and are reported from the beginning of group divergence on the ataxia and paresis scores on day 105 pi to sacrifice on day 177 pi. After animals were sacrificed, neurological lesions were measured in spinal cord and brain sections.

2.3 Cannulation

Cannulation surgeries and vehicle administration were performed in SJL mice to enable comparisons with previous studies using Balb/cJ mice (for details see Meagher et al., 2007, Vichaya et al., 2011). Briefly, mice were anesthetized using 5% isoflurane gas and then maintained on 2% isoflurane gas during stereotaxic surgery. A 33-gauge cannula (PlasticsOne, Roanoke, VA, C315GS-2/SPC) was implanted into the left ventricle. Mice were provided with softened food and acetaminophen treated water (162.5 mg/L) for 48 h prior to being group housed. Vehicle (Ig; Santa Cruz Biotechnology, Inc #SC-2342) was administered 4 h prior to each SDR session in a 2 mL volume at a rate of 1 mL/min via a microinjection pump.

2.4 Social disruption stress

SDR mice were exposed to aggressive male intruders, while Non-SDR mice remained undisturbed in their home cages. Intruders were placed into the home cage at the onset of the dark cycle (17:00 h) for 2 h. The stress procedure occurred for a total of 6 sessions (three consecutive nights, then one night off, followed by three more consecutive nights). The sessions were monitored to ensure that the intruder demonstrated dominant behavior and the residents demonstrated submissive behavior. If intruders failed to attack within 10 min, they were replaced and the session was continued for the remaining 2 h. Although physical contact between intruders and residents occurred, no visible wounds or injuries were noted. This is in agreement with previous studies suggesting that when young mice undergo the SDR stressor, injuries are diminished compared to sessions with older age-matched intruders and residents (Johnson et al., 2006, Vichaya et al., 2011, Young et al., 2013).

2.5 Virus and infection

The BeAn strain of Theiler's virus was obtained from Dr. H.L. Lipton (Department of Microbiology-Immunology, University of Illinois, Chicago, IL) and was propagated in L2 cells (Welsh et al., 1987). Mice were anesthetized with isoflurane and inoculated with 5×10^4 pfu of TMEV or sterile saline in a 20 mL volume into the right mid-parietal cortex (Meagher et al., 2007, Sieve et al., 2004). Inoculation occurred 2 h following the final session of SDR (21:00 h).

2.6 Behavioral assessment

Acute TMEV infection induces a range of sickness behaviors and motor impairment associated with neuroinflammation, which were assessed using multiple measures (Johnson et al., 2006, Johnson et al., 2004, Meagher et al., 2007, Mi et al., 2006, Vichaya et al., 2011, Young et al., 2013). Chronic phase measures were used to assess the clinical progression of TMEV, motor impairment, mechanical nociception, affective pain sensitivity, and memory.

2.6.1 Sucrose preference—Preference for a sweet solution was used to measure sickness-induced anhedonia by giving mice access to a 2% sucrose solution or tap water daily throughout the acute phase then weekly and biweekly during the chronic phase. Sucrose preference was calculated by dividing the intake of sucrose solution over the total amount of fluids consumed (Sieve et al., 2004, 2006, Young et al., 2010).

2.6.2 Body weight—Loss of body weight in the 24 hours following infection has been linked to sickness behavior in animals infected with Theiler's virus (Meagher et al., 2007). During the acute phase, we assessed body weight daily at 09:00 h using a scale sensitive to 0.01g (Scout® Pro Portable Balance, Ohaus, Pine Brook, NJ).

2.6.3 Assessment of sickness behavior and motor impairment—During the acute phase, two measures were used to assess clinical progression of the disease: clinical scores assessing encephalitis and hind limb impairment. These measures were individually analyzed and scored at baseline and on days 1, 4, 7, 14, 21, and 28 pi by a rater blind to experimental condition. Details on these scales have been presented elsewhere (Johnson et al., 2004, Sieve et al., 2004). Briefly, clinical scores for encephalitis-like symptoms during the acute phase were given on a 0–4 scale (0=no behavioral signs of illness, 1=ruffled fur; 2=ruffled fur and slightly hunched posture; 3=ruffled fur, very hunched posture, lethargic; 4=moribund; Sieve et al., 2004).

In addition, the hind limb impairment (HLI) scale assesses weakness and paresis by observing alterations in locomotor function on an inverted grid. A separate score was given to each hind limb (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 muscle tone). These values were then summed to achieve the HLI score with higher scores indicating greater impairment.

After the first month of infection, clinical scores of ataxia and paresis were assessed weekly to measure the progression of the demyelinating chronic phase of disease. For this test, mice were placed on a level grid and both the amount of grip used and their movements were assessed using a 0–6 scale (0=healthy; 1=hind limb weakness; 2=slightly uneven gait; 3=definitely uneven gait; 4=very uneven gait, hunched posture, no righting reflex; 5=uneven gait, hunched posture, no righting reflex, incontinence; 6=moribund; Sieve et al., 2004) with higher scores indicating more disrupted gait and greater clinical progression.

2.6.4 Open field activity—One particular measure used to assess motor impairment, open field behavior, also reflects sickness syndrome when assessed 24 hours following infection (prior to the onset of polio-like symptoms). In addition, during the chronic phase, open field

behavior reflects motivational processes as well as demyelination-induced motor impairments. Thus, a reduction in open field behavior can be interpreted as reflecting theoretically distinct constructs of sickness behavior, motivational processes, and motor impairments at different stages in the disease progression.

Open field vertical and horizontal activity were assessed in SJL mice using six optical beam activity monitors (Model RXYZCM-16, Omnitech Electronics, Columbus, OH) equipped with two banks of eight photocells on each wall. The boxes were interfaced with a digital-multiplexor and Versamax software (Model DCM-4, Omnitech Electronics, Columbus, OH). Mice were habituated to the chambers for 60 min prior to baseline data collection. Test sessions were conducted weekly in the dark beginning at 17:00 h for 30 min with white noise (64 dB) to mask extraneous disturbances. Activity was assessed through breaking of optical beams.

2.6.5 Stride length—Footprint stride length and spread were assessed weekly during the chronic phase (for details see Johnson et al., 2006). Briefly, hind paws were painted with blue finger paint while forepaws were painted with red finger paint. Mice walked down a 2.5'' by 36'' runway lined with paper to record limb placement. Decreased stride length and spread reflect increased TMEV-induced neuroinflammation and demyelination (McGavern et al., 1999, McGavern et al., 2000).

2.6.6 Rotarod—Motor coordination was assessed weekly during the chronic phase using the rotarod test. Previous studies in SJL mice indicate that performance on this test is impaired during the chronic phase of TMEV infection (McGavern et al., 1999, Sieve et al., 2004). Prior to any manipulation, mice were trained for 3 days on the rotarod and baseline performance was measured. Testing was conducted every week following the onset of clinical signs. During testing, mice were placed on a rod located 20 cm above a padded platform rotating at 6 rpm, and rotation speed was increased by 3 rpm (9, 12, 15, 18, 21, 24, 27, and 30 rpm) every 30 s. The latency and speed at which the subject fell from the rod was recorded, with shorter times reflecting impaired balance.

2.7 Measures of cognition and pain

2.7.1 Mechanical sensitivity—Mechanical sensitivity was assessed prior to infection and weekly throughout the acute and chronic phases (for details see Meagher et al., 2007). Prior to testing, mice were habituated to the apparatus for 20 min. Mice were tested in transparent circular chambers positioned on a raised mesh screen (2 mm gauge) where nylon filaments (von Frey monofilaments; Stoelting, Wooddale, IL) were applied to the hind paw to determine their withdrawal threshold. Starting with the smallest filament pressure, each filament was applied to the left and right hind paws in an ABBA manner. Mice received three trials using von Frey filaments with differing pressures ranging from 0.008 – 2.0 grams in ascending, descending, and ascending order (Dixon, 1980). A lower von Frey filament force indicates a lower mechanical withdrawal threshold and therefore increased mechanical hypersensitivity.

2.7.2 Fear conditioning—Fear conditioning was used to determine whether infection and stress altered affective pain processing during the chronic phase of the disease (King et al., 1996, McLemore et al., 1999, Meagher et al., 2001) and was assessed over days 159-163 pi. The fear-conditioning apparatus consisted of a gray metal square conditioning chamber (30 × 30 × 30 cm) with one clear Plexiglas wall to allow for video recording (Coulbourn instruments, PA, USA). The grid floor of the chamber was wired to a shock generator and a scrambler (Coulbourn instruments, PA, USA) that was used to administer a 2 mA footshock. The chambers were positioned in a sound attenuated test cubicle and were illuminated by a house light. On day one, mice were pre-exposed to the Colburn Chamber for 5 min and immediately returned to their home cage. On day two, mice were conditioned to the context by being placed in the Coulbourn Chamber for 2 minutes where they were exposed to 2 tone-shock pairings consisting of a 30 sec tone that co-terminated with a 2 mA shock with intervening 30 sec ITIs. Thirty seconds following the second shock subjects were removed from the chamber. Twenty-four hours later, on day three, the subjects had baseline freezing scores recorded (pre-cue) and were returned to the original chamber in which the conditioning took place under original conditions for 5 minutes to test context conditioning (context). In order to test cue conditioning to the CS, one hour later the subjects were returned to the chamber that was altered in appearance, floor surface, and scent. They were allowed to explore the modified chamber for two minutes and were then exposed to the conditioned tone for three minutes (CS). Video recordings of each session were divided into 5 sec bins for all three phases (pre-CS, context, and CS) and were scored by blinded observers for conditioned fear (freezing: defined as the absence of all movement except for those needed for normal respiration). The percentage of time freezing was calculated by the number of bins in which the animal froze divided by the total number of bins comprising that phase. If mice perceived the stimulus as more painful, they showed enhanced levels of conditioned freezing (King et al., 1996, McLemore et al., 1999, Meagher et al., 2001).

2.7.3 Novel object recognition—The novel object recognition test assessed whether stress exacerbated TMEV infection-induced hippocampal-dependent memory impairment during the chronic phase of the disease. In the novel object recognition task (Ennaceur and Delacour, 1988), mice were individually exposed to two identical objects in a novel test chamber (8"x10") for 10 minutes. The time spent exploring each object was recorded using a video camera. After a one-hour retention interval, as determined using a pilot study of age-matched control animals indicating that object memory persisted for this duration, memory for the objects was assessed by returning the mouse to the test chamber with two items: one familiar object and one novel object. The animal was allowed to explore the two items for 10 minutes while being recorded. Objects were randomized and counterbalanced across animals. Between trials, the objects and chamber were cleaned with 70% ethanol to remove any cues. Raters blind to experimental condition scored videotapes for the amount of time spent interacting with each object. A recognition index was calculated (Antunes and Biala, 2012, Broadbent et al., 2010) and was defined as the amount of time interacting with the new object over the total amount of time spent exploring both objects. A recognition index of 0.5 indicates no memory for the familiar object.

2.8 Immunological and histological measures

2.8.1 Antibody responses to Theiler's virus—Radioimmunoassays (RIAs) were used to measure serum antibodies against Theiler's virus (whole virus, identical to that used for infection) using previously described procedures (Dolimbek et al., 2002, Sieve et al., 2004, Young et al., 1983). RIA uses radiolabeled protein A, which binds to the Fc portion of immunoglobulin, and allows the level of radioactivity (counts per minute, cpm) to be equated with antibody level (Dolimbek et al., 2002, Young et al., 1983). Antibody levels were assessed at days 25, 50, 125, and 177 pi to assess the acute and chronic phases as well as the intervening time. Blood draws were conducted via the femoral vein on days 25, 50, and 125 pi and by exsanguination on day 177 pi.

2.8.2 CNS tissue collection and preparation—Mice were deeply anesthetized and sacrificed at day 177 pi during gravity perfusion with phosphate-buffered saline followed by 4% paraformaldehyde. Brains and vertebral columns (containing intact spinal cord) were then removed and embedded in paraffin blocks and stored at 4°C until sectioning. The tissues were then sectioned on a microtome and mounted on slides; brains were sectioned coronally at 4 levels while the spinal cord was sectioned transversely at 6 levels (see Campbell et al., 2001 for further detail). Paraffin was removed from sections using xylene and rehydrated using graded alcohol baths. Tissues were then processed for routine hematoxylin and eosin (H&E) and Weil's myelin stains.

2.8.3 Histology—Tissues were stained to evaluate diffuse accumulation of mononuclear inflammatory cells within the parenchyma (referred to as microgliosis), accumulation of inflammatory cells around blood vessels (perivascular cuffing), accumulation of inflammatory cells in the meninges (meningitis), spongy appearance of the parenchyma (status spongiosis), and demyelination. Lesions of the spinal cord and brain were scored by raters blind to condition. Spinal cord and brain lesions were assessed using previously described methods (Young et al., 2010). Briefly, meningitis was determined using the length of inflamed meninges (μm) divided by the total length of the meninges. In area CA1 of the hippocampus, lesions were similarly determined by measuring the length of neuronal degeneration (i.e. the absence of pyramidal cells) in area CA1 divided by the total length of the pyramidal cell layer in area CA1 using ImageJ version 1.47 (National Institutes of Health, 2013). Perivascular cuffing and microgliosis were determined by measuring the total area of inflammation (μm^2) divided by the total area of the section. The periaqueductal gray, locus coeruleus, and rostral ventromedial medulla were investigated for their association with affective pain while the hippocampal CA1 region was investigated for its association with cognition.

2.9 Statistical Analyses

Data are presented as mean $\pm$ SEM. One-way analyses of variance (ANOVAs) were used to evaluate differences between infection and SDR groups while repeated measures ANOVAs and repeated measures analysis of covariance (ANCOVAs) with baseline scores entered as the covariates were used to evaluate differences between groups over time, when appropriate. Follow-up analyses were conducted using Fisher's LSD for differences between

groups and post hoc mean comparisons for interaction effects and where appropriate. Analyses were carried out using SPSS Statistics 20 (Chicago, IL).

3. Results

3.1 Acute phase

3.1.1 Measures of sickness behavior were greater in infected alone and stressed and infected mice—Prior studies have shown changes in body weight, sucrose preference, activity, mechanical sensitivity, and open field behavior one day after TMEV infection in Balb/cJ mice (Meagher et al., 2007, Vichaya et al., 2011), indicative of cytokine-induced sickness syndrome (Barak et al., 2002a, Barak et al., 2002b, Dantzer and Kelley, 2007, Pollak et al., 2000, Watkins et al., 1995). Change scores between day 1 pi and baseline were assessed for body weight (Fig. 2A), sucrose preference (Fig. 2B), vertical activity (Fig. 2C), horizontal activity (Fig. 2D), and mechanical sensitivity (Fig. 2E). Using one-way ANOVAs, main effects for group were found for changes in body weight, $F(2, 15) = 11.45$, $p = .001$, and horizontal activity, $F(2, 15) = 9.43$, $p = .002$, with Fisher's LSD showing significant effects of infection on lowering both body weight (p values $< .005$) and horizontal activity (p values $< .01$). There was also a main effect of group for vertical activity, $F(2, 15) = 4.05$, $p = .039$. Fisher's LSD indicated that SDR infected animals showed significantly decreased vertical activity compared to the Non-SDR infected group ($p = .012$). No main effects of group in the remaining ANOVAs were seen in sucrose preference ($p = .309$) or mechanical sensitivity ($p = .981$).

3.1.2 Clinical scores were greater in infected mice—As would be anticipated, infected mice exhibited symptoms of encephalitis indicated by their higher clinical scores (Fig. 3A). A repeated measures ANCOVA confirmed a main effect of group, $F(2, 13) = 61.772$, $p < .001$, with Fisher's LSD revealing that SDR and Non-SDR infected groups had worse clinical scores than Non-SDR uninfected animals (both $ps < .001$).

3.1.3 Hind limb impairment (HLI) ratings were greater in infected mice—Hind limb impairment, a measure of motor function recorded in conjunction with clinical scores, was greatest in infected animals (Fig. 3B). A repeated measures ANCOVA of acute phase HLI confirmed a main effect of group, $F(2, 13) = 105.25$, $p < .001$, with Fisher's LSD revealing the infected groups showed significantly greater hind limb impairment than the Non-SDR uninfected controls (both $ps < .001$).

3.1.4 Open field activity was reduced during early acute phase in infected mice—Reduced activity reflects the effects of Theiler's infection on sickness behavior as well as motor function (Johnson et al., 2006, McGavern et al., 1999, Meagher et al., 2007, Young et al., 2010). A repeated measures ANCOVA of vertical activity (Fig. 3C) during the acute phase revealed a significant main effect of time, $F(4, 52) = 4.97$, $p = .002$, and an interaction between time and group, $F(8, 52) = 4.73$, $p < .001$. Mean comparisons revealed that vertical activity generally increased over time with the greatest increase in the infected groups. This increase is likely due to the increase in activity between days 1 and 4 pi with a maximum difference at day 1 pi, $F(2, 15) = 4.93$, $p = .023$, with infected groups exhibiting

reduced vertical activity (both $ps < .04$) followed by an increase in activity in these groups by day 4 pi (Fig. 3C) indicating recovery of activity. Following day 4 pi, infected groups showed slightly decreased activity through the acute phase while the Non-SDR uninfected controls showed an increase in vertical activity through the same phase.

There was a similar effect of decreased horizontal activity during the acute phase (Fig. 3D). A repeated measures ANCOVA during the acute phase confirmed a significant effect of time, $F(4, 52) = 3.78, p = .009$, and an interaction between time and group, $F(8, 52) = 6.54, p < .001$. Fisher's LSD showed that on day 1 pi infected mice (SDR and Non-SDR infected) had decreased horizontal activity compared to Non-SDR uninfected controls and showed an increase in activity for infected groups by day 4 pi.

3.2 Chronic phase

3.2.1 Behavioral data—Chronic infection led to impairment on three measures of motor function (rotarod, ataxia/paresis, and stride length), but it had no effect on spontaneous activity (Fig. 4B). Although horizontal activity decreased over time, spontaneous activity during the chronic phase was not sensitive to stress or infection.

A repeated measures ANCOVA conducted on rotarod times over days post-infection with baseline rotarod time as a covariate revealed a significant interaction between group and time, $F(16, 104) = 2.13, p = .012$, for rotarod performance during the chronic phase (Fig. 4A) but no main effect of group, $F(2, 13) = 0.74, p = .497$. A trend analysis across the chronic phase revealed a significant interaction between group and time, $F(2, 13) = 6.29, p = .012$ that was best fit using a quadratic model. This infection by time interaction was driven by the Non-SDR uninfected mice, which showed moderately increased times on days 107-114 followed by decreased times and then a significant increase in rotarod times on day 161. The increase in time on day 161 is supported by an ANCOVA conducted on day 161 which revealed a main effect of group, $F(2, 14) = 4.102, p = .04$. Fisher's LSD indicated that the SDR infected mice showed decreased rotarod times compared to Non-SDR uninfected controls ($p = .017$) and the Non-SDR infected mice showed a trend towards having significantly reduced rotarod times compared to the Non-SDR uninfected controls ($p = .053$). Due to the possible effect of infection, post hoc mean comparisons were conducted on group means comparing infected (SDR infected and Non-SDR infected) and uninfected mice for day 161. Results indicated that chronic infection led to lower rotarod times compared to the Non-SDR uninfected group, $F(1, 16) = 8.42, p = .011$, during late disease (see inset of Fig 4A).

A repeated measures ANCOVA confirmed main effects of time, $F(9, 117) = 9.1, p < .001$, and group, $F(2, 13) = 61.78, p < .001$, as well as an interaction between time and group, $F(18, 117) = 7.53, p < .001$, for clinical ataxia and paresis (Fig. 4B). Mean comparisons revealed that infected subjects exhibited worsening symptoms over time compared to uninfected subjects. Although an overall effect of stress during the chronic phase was not observed, by the final time point (day 170 pi), the SDR infected group exhibited greater ataxia and paresis scores than the Non-SDR infected group, $F(2, 14) = 104.48, p < .001$.

Hind stride length was decreased in the Non-SDR infected group (Fig. 4C), indicating enhanced motor impairment. A repeated measures ANOVA for hind stride length showed main effects of time, $F(11, 154) = 2.32, p = 0.012$, and group, $F(2, 14) = 5.40, p = .018$, as well as an interaction between time and group, $F(22, 154) = 1.75, p = .024$. Mean comparisons revealed the Non-SDR infected group had shorter hind stride length than the two other experimental groups with the Non-SDR uninfected group showing increased stride length over time (Fig. 3C). Similar results were observed for fore stride length (data not shown) with the Non-SDR infected group having a shorter stride length.

For horizontal activity during the chronic phase, a significant effect of time was observed, $F(4, 52) = 2.62, p = .045$ (Fig. 4D). Mean comparisons indicated an overall decrease in horizontal activity as the chronic stage progressed. After controlling for baseline activity levels (taken post-SDR but pre-infection; using an ANCOVA) no significant effects were found for vertical activity (Fig. 4E). This lack of significance after controlling for the baseline covariate may be due to baseline scores that were already increased by the prior SDR and led to a pre-existing difference that minimized any difference in the chronic phase as removing the covariate resulted in a significant main effect of time ($p = .021$).

3.2.5 Mechanical sensitivity was lower in stressed and infected mice—Stress prior to infection and infection alone were associated with reduced mechanical threshold, indicating greater sensitivity to mechanical nociception (Fig. 5A). A repeated measures ANCOVA revealed effects of time, $F(5, 65) = 9.727, p < .001$, and group, $F(2, 13) = 15.237, p < .001$, as well as an interaction between time and group, $F(10, 65) = 3.657, p = .001$. Fisher's LSD revealed that the SDR infected group had significantly lower mechanical threshold than the two other groups (both $ps < .005$). One-way ANOVAs of specific days revealed a significant effect of group, $F(2, 14) = 8.35, p = .004$, on day 105 pi with Non-SDR infected showing the highest threshold and on day 147, $F(2, 14) = 6.84, p = .008$, with infected groups showing significantly lower threshold. Significant main effects of group were also found on days 133, 166, and 168, all $F(2, 14) \geq 8.3$, all $ps < .01$, with SDR infected mice exhibiting the greatest sensitivity.

3.2.6 Fear conditioning was enhanced in the stressed and infected mice—An ANOVA conducted on baseline levels of freezing behavior showed infected mice froze more prior to shock, $F(2, 14) = 3.95, p = .044$ (data not shown), which may reflect infection related changes in motor activity or anxiety/depression. Given these differences, analyses of context freezing controlled for baseline differences by subtracting pre-cue freezing from context freezing. As indicated in Fig. 5B, stress prior to infection was associated with increased freezing behavior in response to the chamber they were previously conditioned to, a measure of the affective impact of footshocks during the chronic phase of the disease. A one-way ANOVA revealed a significant effect of group, $F(2, 14) = 3.75, p = .05$, with Fisher's LSD showing the SDR infected group froze more than the Non-SDR infected ($p = .044$) and Non-SDR uninfected ($p = .027$) groups.

3.2.7 Novel object recognition was impaired in stressed and infected mice—Stress prior to infection had a detrimental effect on the memory consolidation of mice (Fig. 5C). A one-way ANOVA showed a trend towards a significant group difference ($p = .065$)

and Pearson's correlation between group and novel object recognition scores suggest a linear relationship between groups, $r = -.554$, $p = .021$. This was confirmed by a significant linear trend in which the Non-SDR uninfected group exhibited the greatest amount of learning and the SDR infected group showed the least, $F(1, 14) = 6.35$, $p = .024$. Fisher's LSD revealed a significant difference between the Non-SDR uninfected group and the SDR infected group ($p = .024$).

3.3 Antibody responses to Theiler's virus was greater in infected mice

As indicated in Fig. 6, higher levels of antibodies were related to the TMEV infection. Repeated measures ANOVA confirmed this with significant main effects of time, $F(3, 42) = 48.57$, $p < .001$, and group, $F(2, 14) = 19.42$, $p < .001$, as well as an interaction effect between time and group, $F(6, 42) = 7.42$, $p < .001$, for antibodies to TMEV (Fig. 6). Mean comparisons for group revealed an effect of infection, with infected groups exhibiting greater TMEV titer levels than the uninfected controls (both $ps < .001$) that increased over time. On day 125 pi, a follow-up one-way ANOVA revealed a significant effect of group, $F(2, 17) = 10.747$, $p = .001$, with Fisher's LSD showing the SDR infected group having decreased antibodies to TMEV than the Non-SDR infected group ($p = .024$) but had more antibodies than the Non-SDR uninfected group ($p = .044$).

3.4 Histology

3.4.1 Spinal cord lesions were greater in infected mice—Infection was associated with more severe spinal cord inflammation and demyelination (Fig. 7A-C). One-way ANOVAs revealed significant differences in the length of meningitis, $F(2, 14) = 9.92$, $p = 0.002$ (Fig. 7A), and area of microgliosis, $F(2, 14) = 5.2$, $p = .020$ (Fig. 7B), and demyelination, $F(2, 14) = 4.84$, $p = .025$ (Fig. 7C). For all three lesion categories, Fisher's LSD revealed an effect of infection, with both infected groups exhibiting significantly greater meningitis than the Non-SDR uninfected group (all $ps < .05$).

Pearson's correlations showed spinal cord meningitis, microgliosis, and demyelination were positively correlated with the amount of antibodies on day 170 pi, all $rs(17) > .7$, all $ps < .005$. In addition, all three measures of spinal cord inflammation were positively correlated with ataxia and paresis scores throughout the chronic phase, all $rs(17) > .52$, all $ps < .05$.

3.4.2 Brain lesions were greater in infected mice—Cognitive impairments, including spatial memory deficits (Ruet et al., 2013), and hippocampal structural deficits (Roosendaal et al., 2010) have been identified in both humans with multiple sclerosis and animal models (Ziehn et al., 2010). Infection was associated with greater lesions, defined as the length of neuronal degeneration, in area CA1 of the hippocampus (Fig. 7D; for examples of lesions, see Fig. 8). A one-way ANOVA confirmed group differences in the area of hippocampal lesions, $F(2, 15) = 5.11$, $p = .023$. Fisher's LSD revealed that Non-SDR uninfected mice showed significantly fewer hippocampal lesions compared to Non-SDR infected and SDR infected mice ($ps = .039$ and $.008$, respectively), which did not differ between themselves ($p = .492$). Due to the similarity of infected groups, they were combined in a one-way ANOVA that showed hippocampal lesions were related to novel object recognition scores $F(2, 15) = 3.909$, $p = .047$, such that mice with no lesions showed

significantly greater object recognition than the mice with few (<10% lesioned area, $p = .039$) or multiple (>10% lesioned area, $p = .034$) lesions which did not differ between themselves ($p = .940$). The location of lesions in the anterior or posterior halves of the hippocampus had no significant effect on novel object recognition scores, $F(1,18) = .643$, $p = .434$. Correlations between hippocampal lesions and spinal cord lesions were significant for meningitis ($r = .54$, $p = .016$) and demyelination ($r = .48$, $p = .03$) and trended towards significant for microgliosis ($r = .36$, $p = .083$). A one-way ANOVA for hindbrain lesions in the periaqueductal gray, locus coeruleus, and rostral ventromedial medulla found no significant group effect ($p > .05$).

4.0 Discussion

The present study examined the effects of social disruption stress on TMEV disease course in the highly susceptible SJL mouse strain. Social disruption stress has been shown to exacerbate TMEV-induced neuroinflammation and disease severity in stress-reactive Balb/cJ mice (Johnson et al., 2006, Johnson et al., 2004, Meagher et al., 2007, Vichaya et al., 2011). Here we examined whether social stress administered prior to infection would exacerbate the chronic phase of disease in SJL mice. To facilitate comparisons with prior studies, similar behavioral and histological measures of disease course were used in the present study. To advance beyond these measures, we included new assessments of the affective component of pain and hippocampal-dependent memory consolidation during the chronic phase of disease. Below we relate our findings to previous research before focusing on our novel and clinically significant pain and memory findings.

4.1 Relation to previous TMEV studies

Our laboratory has previously shown that SJL mice show increased sensitivity to TMEV infection following SDR during the acute phase of infection (Young et al., 2013). SDR was found to increase CNS viral load and exacerbated disease by suppressing virus-specific T cell responses in the CNS. The present study is the first to assess whether the deleterious effects of SDR during acute infection lead to disease exacerbation during the chronic phase. To our surprise, the adverse behavioral and immunological effects of SDR previously observed in BALBc/J mice were not consistently observed in SJL mice during the chronic phase. However, we did find effects of SDR and infection on the nociceptive and affective dimensions of pain as well as on memory.

Consistent with prior studies, TMEV infection induced behavioral signs of sickness behavior and motor impairment in SJL mice. Infection-related decreases in body weight (Johnson et al., 2006, Johnson et al., 2004, Meagher et al., 2007, Vichaya et al., 2011, Young et al., 2013) and exploratory behavior (horizontal activity; Meagher et al., 2007, Vichaya et al., 2011) were observed 24 hours post-infection, suggesting that acute infection induces sickness syndrome in SJL mice (Meagher et al., 2007, Vichaya et al., 2011). At day 1 pi, infected mice showed clinical signs of infection (Vichaya et al., 2011) and throughout the acute phase, they showed increased hind limb impairment (Johnson et al., 2006, Johnson et al., 2004, Meagher et al., 2007). Infection alone increased antibodies to virus and inflammatory CNS lesions, and decreased motor coordination on the rotarod task

(McGavern et al., 1999, McGavern et al., 2000, Sieve et al., 2004). Although stress prior to infection did not significantly alter acute phase clinical scores, during the chronic phase stress and infection led to increases in clinical scores on day 170 pi (Sieve et al., 2004), and enhanced mechanical sensitivity (Lynch et al., 2008a, Lynch et al., 2008b). In addition, infection led to greater inflammation and demyelination in the spinal cord with no effect seen for SDR stress. This is consistent with Young et al. (2010) who found no change in spinal inflammation to restraint stress in male SJL/J mice. That brain lesions were correlated with spinal cord lesions provides evidence that lesions in the brain were the result of TMEV infection. Due to experimental design limitations and planned tests of pain and cognitive impairments, we did not include additional measures of stride length and open field activity. Thus, it is possible that additional effects on these measures might have been observed if testing had been carried out past days 139 to 170.

While several findings are consistent with prior results in Balb/cJ mice, we did not observe changes in sickness behavior during acute infection on the sucrose preference (anhedonia) or mechanical sensitivity (allodynia) tasks, suggesting that SJL mice may experience less severe neuroinflammation during early infection. Moreover, we did not observe infection- or stress-related differences in vertical or horizontal activity during the chronic phase of the disease (McGavern et al., 1999, Sieve et al., 2006). While differences in the virulence of the virus and stressors across studies may explain this discrepancy—Sieve et al. (2004) used restraint stress while our study used social stress—differences in study design may also play a role. Sieve and colleagues (2004) sampled activity on days 57, 77, and 105 pi while the present study sampled over days 112, 119, 124, 132, and 139 pi. By delaying assessment until the advanced stage of the disease, the present study allowed for greater disease progression and likely a ceiling effect with all groups showing decreased activity (see Fig. 4b). We have previously shown that SDR prior to infection not only decreased horizontal and vertical activity during the chronic phase, it also reduced stride length in Balb/cJ mice (Johnson et al., 2006, Meagher et al., 2007), behaviors which have been correlated with TMEV-induced inflammation and demyelination (McGavern et al., 1999). Unexpectedly, the SDR infected SJL mice in the present study showed longer stride length compared to the Non-SDR infected mice. In isolation this might be interpreted as a protective effect, however this seems unlikely as it does not correspond to a reduction in inflammatory lesions. In contrast to the effects of SDR on Balb/cJ mice, we did not observe a robust increase in serum TMEV antibody levels in SJL mice. Although post hoc analyses revealed a small reduction in antibody levels in SDR infected mice compared to the Non-SDR infected mice on day 125 pi, there was no difference on day 170 pi, which is similar to the negative effects of restraint stress on TMEV antibodies observed in male SJL mice (Sieve et al., 2004). Other mechanisms, such as opioids, which have been implicated in the nociceptive dimension of pain in TMEV models (Lynch et al., 2008a), have also been shown to play a role in social disruption stress (Chaijale et al., 2013, Van'T Veer and Carlezon Jr, 2013), and fear conditioning and memory (Bryant et al., 2009). Thus, the present findings may be due, in part, to alterations in the endogenous opioid system.

4.2 Cognitive impairments: TMEV- and stress-induced decreases in memory consolidation with infection-related increase in CA1 lesions

Though cognitive deficits are pervasive and significant symptoms of MS, the mechanisms mediating cognitive dysfunction remain understudied. A few experiments investigating the effects of acute TMEV infection have shown infection-related impairments in spatial memory during the polio-like acute phase of the disease using hippocampal-dependent spatial memory tasks (Buenz et al., 2006, Howe et al., 2012a, Howe et al., 2012b). However, the spatial memory tasks (e.g., Morris Water Maze) used in these acute phase studies have high motor demands and cannot be used during the demyelinating chronic phase. Therefore, the present study utilized a hippocampal-dependent novel object recognition task, which minimizes motor demands and thus may provide a valid measure of memory during late disease. Using this task, we found infection- and stress-induced memory deficits during the chronic phase of TMEV infection in highly-TMEV susceptible SJL mice. In addition, we found that diminished novel object recognition scores across groups were associated with lesions in area CA1 of the hippocampus and that infection was related to greater CA1 lesions in the hippocampus. These memory impairments are consistent with previous work indicating that mice with hippocampal damage, specifically of the CA1 region (D'Hooge and De Deyn, 2001, McHugh et al., 1996), are unable to form spatial memories (Andersson et al., 1993, Cho et al., 1999). Taken together, these findings suggest that the novel object recognition test may be used during the chronic phase of TMEV infection to study the neurobiological mechanisms contributing to cognitive impairments in MS.

4.3 SDR-induced enhancement of the nociceptive and affective dimensions of pain

Approximately 50% of patients with Multiple Sclerosis experience chronic pain, but relatively little research has addressed this issue. Pre-clinical research has begun to study the nociceptive dimension of MS pain using the TMEV model. Notably, Lynch et al. (2008a, 2008b) found TMEV infection induces mechanical allodynia and thermal pain sensitivity. The present study, using the traditional up/down method of allodynia testing, observed heightened allodynia in SDR infected mice compared to the Non-SDR infected mice. Our less robust findings than Lynch et al. (2008a, 2008b) may be due to differences in allodynia assessment methods and sample sizes between studies. Importantly, our study adds to the literature by utilizing a fear conditioning task to assess the affective and motivational dimension of pain. In line with the nociceptive results, this measure of aversion showed that the SDR infected mice froze more than their Non-SDR counterparts, indicating that the shock was experienced as more aversive.

Of the two dimensions of pain – sensory and affective – nociceptive withdrawal reflexes are thought to reflect sensory processing at the level of the spinal cord (Johansen et al., 2001, King et al., 2009, Vierck et al., 2008). However, clinical studies of pain in humans focus mainly on the affective or “unpleasant” component of pain, which reflects supraspinal processing of the pain signal in brain regions involved in affective and motivational processes (King et al., 2009). In this study, we showed that the combination of stress and Theiler’s infection exacerbated both the nociceptive and affective dimensions of pain indicated by enhanced mechanical sensitivity and fear conditioning, respectively. Thus, the contextual fear conditioning test, and perhaps other associative learning measures of the

affective experience of pain (King et al., 2009), may be used to study the mechanisms mediating MS-induced pain.

4.4 Stress-induced priming

Across a variety of models, stress exposure can potentiate neuroinflammation in the CNS exacerbating symptomatology (Deak et al., 2005, Frank et al., 2007, Frank et al., 2012, Johnson et al., 2002a, Johnson et al., 2002b, Karelina et al., 2009, Norman et al., 2010, Perry and Holmes, 2014, Weil et al., 2008). In the present model, the presumed mechanism involves a social stress-induced increase in central and peripheral IL-6 response to TMEV (Meagher et al., 2007), which primes the CNS to an enhanced innate immune response (Vichaya et al., 2011) and disrupts the adaptive CNS immune response (Young et al., 2013). The resultant neuroinflammation can exacerbate behavioral impairments in both the acute and chronic phases of disease (Johnson et al., 2014, Johnson et al., 2006, Johnson et al., 2004). While this process may underlie our SDR-related exacerbations in SJL mice, including enhanced pain behavior and memory consolidation, it should be noted that these effects may be differentially expressed in mouse strains as many strains differ in their sensitivity to stress (Anisman et al., 2001, Belzung et al., 2001, Griebel et al., 2000). Future studies are needed to further examine this mechanism.

4.5 Summary and implications

Animal models are needed to systematically investigate factors that modulate MS disease severity, including the effects of social stressors on the development of pain and cognitive deficits. The present study found exposure to social stress prior to infection with TMEV increased pain and impaired hippocampal-dependent memory consolidation during the demyelinating chronic phase of disease in SJL mice. Although our results should be considered preliminary, they suggest that the TMEV model may be used to investigate the mechanisms mediating pain and cognitive impairments in MS, and the role of social stress-induced neuroinflammation in exacerbating these symptoms. Future research is needed to elucidate central mechanisms mediating the adverse effects of social stress on disease course, which may lead to interventions designed to mitigate pain and cognitive deficits in humans with MS.

Acknowledgements

This research was supported by NIH/NINDS R01-NS060822 awarded to MWM and CJW and a Heep Fellowship to HRL. The authors would also like to thank Erich Spoor for help with brain histology.

Glossary

MS	Multiple sclerosis
TMEV	Theiler's murine encephalomyelitis virus
CNS	Central nervous system
SDR	Social disruption
pnd	Day post-natal

pi	Post-infection
HLI	Hind limb impairment
H&E	Hematoxylin and eosin
dB	Decibels

References

- Ackerman KD, Heyman R, Rabin BS, Anderson BP, Houck PR, Frank E, et al. Stressful life events precede exacerbations of multiple sclerosis. *Psychosom Med*. 2002; 64:916–20. [PubMed: 12461197]
- Amato MP, Ponziani G, Pracucci G, Bracco L, Siracusa G, Amaducci L. Cognitive impairment in early-onset multiple sclerosis. Pattern, predictors, and impact on everyday life in a 4-year follow-up. *Arch Neurol*. 1995; 52:168–72. [PubMed: 7848126]
- Amato MP, Ponziani G, Siracusa G, Sorbi S. Cognitive dysfunction in early-onset multiple sclerosis: a reappraisal after 10 years. *Arch Neurol*. 2001; 58:1602–6. [PubMed: 11594918]
- Andersson T, Schwarcz R, Löve A, Kristensson K. Measles virus-induced hippocampal neurodegeneration in the mouse: A novel, subacute model for testing neuroprotective agents. *Neurosci Lett*. 1993; 154:109–12. [PubMed: 8361621]
- Anisman H, Hayley S, Kelly O, Borowski T, Merali Z. Psychogenic, neurogenic, and systemic stressor effects on plasma corticosterone and behavior: Mouse strain-dependent outcomes. *Behav Neurosci*. 2001; 115:443–54. [PubMed: 11345969]
- Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process*. 2012; 13:93–110. [PubMed: 22160349]
- Barak O, Goshen I, Ben-Hur T, Weidenfeld J, Taylor AN, Yirmiya R. Involvement of brain cytokines in the neurobehavioral disturbances induced by HIV-1 glycoprotein120. *Brain Res*. 2002a; 933:98–108. [PubMed: 11931854]
- Barak O, Weidenfeld J, Goshen I, Ben-Hur T, Taylor AN, Yirmiya R. Intracerebral HIV-1 glycoprotein 120 produces sickness behavior and pituitary-adrenal activation in rats: Role of prostaglandins. *Brain Behav Immun*. 2002b; 16:720–35. [PubMed: 12480502]
- Beard S, Hunn A, Wight J. Treatments for spasticity and pain in multiple sclerosis: a systematic review. *Health Technol Assess*. 2003; 7:iii. ix-x, 1-111. [PubMed: 14636486]
- Belzung C, El Hage W, Moindrot N, Griebel G. Behavioral and neurochemical changes following predatory stress in mice. *Neuropharmacol*. 2001; 41:400–8.
- Broadbent NJ, Gaskin S, Squire LR, Clark RE. Object recognition memory and the rodent hippocampus. *Learn Mem*. 2010; 17:5–11. [PubMed: 20028732]
- Brown RF, Tennant CC, Dunn SM, Pollard JD. A review of stress-relapse interactions in multiple sclerosis: Important features and stress-mediating and -moderating variables. *Mult Scler*. 2005; 11:477–84. [PubMed: 16042233]
- Brown RF, Tennant CC, Sharrock M, Hodgkinson S, Dunn SM, Pollard JD. Relationship between stress and relapse in multiple sclerosis: Part I. Important features. *Mult Scler*. 2006a; 12:453–64. [PubMed: 16900759]
- Brown RF, Tennant CC, Sharrock M, Hodgkinson S, Dunn SM, Pollard JD. Relationship between stress and relapse in multiple sclerosis: Part II. Direct and indirect relationships. *Mult Scler*. 2006b; 12:465–75. [PubMed: 16900760]
- Bryant RA, Creamer M, O'Donnell M, Silove D, McFarlane AC. A Study of the Protective Function of Acute Morphine Administration on Subsequent Posttraumatic Stress Disorder. *Biological Psychiatry*. 2009; 65:438–40. [PubMed: 19058787]
- Buenz EJ, Rodriguez M, Howe CL. Disrupted spatial memory is a consequence of picornavirus infection. *Neurobiol Dis*. 2006; 24:266–73. [PubMed: 16919964]

- Campbell T, Meagher MW, Sieve A, Scott B, Storts R, Welsh TH, et al. The effects of restraint stress on the neuropathogenesis of Theiler's virus infection: I. Acute disease. *Brain Behav Immun*. 2001; 15:235–54. [PubMed: 11566048]
- Chaijale NN, Curtis AL, Wood SK, Zhang XY, Bhatnagar S, Reyes BA, et al. Social stress engages opioid regulation of locus coeruleus norepinephrine neurons and induces a state of cellular and physical opiate dependence. *Neuropsychopharmacology*. 2013; 38:1833–43. [PubMed: 23660707]
- Cho YH, Friedman E, Silva AJ. Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. *Behav Brain Res*. 1999; 98:77–87. [PubMed: 10210524]
- Compston A, Coles A. Multiple sclerosis. *Lancet*. 2008; 372:1502–17. [PubMed: 18970977]
- Covey TJ, Zivadinov R, Shucard JL, Shucard DW. Information processing speed, neural efficiency, and working memory performance in multiple sclerosis: differential relationships with structural magnetic resonance imaging. *J Clin Exp Neuropsychol*. 2011; 33:1129–45. [PubMed: 22047454]
- D'Hooze R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev*. 2001; 36:60–90. [PubMed: 11516773]
- Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain Behav Immun*. 2007; 21:153–60. [PubMed: 17088043]
- Deak T, Bordner KA, McElderry NK, Barnum CJ, Blandino P Jr, Deak MM, et al. Stress-induced increases in hypothalamic IL-1: A systematic analysis of multiple stressor paradigms. *Brain Res Bull*. 2005; 64:541–56. [PubMed: 15639551]
- Dixon WJ. Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol*. 1980; 20:441–62. [PubMed: 7387124]
- Dolimbek BZ, Jankovic J, Atassi MZ. Cross reaction of tetanus and botulinum neurotoxins A and B and the boosting effect of botulinum neurotoxins A and B on a primary anti-tetanus antibody response. *Immunol Invest*. 2002; 31:247–62. [PubMed: 12472183]
- Ehde DM, Osborne TL, Hanley MA, Jensen MP, Kraft GH. The scope and nature of pain in persons with multiple sclerosis. *Mult Scler*. 2006; 12:629–38. [PubMed: 17086910]
- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res*. 1988; 31:47–59. [PubMed: 3228475]
- Frank MG, Baratta MV, Sprunger DB, Watkins LR, Maier SF. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. *Brain Behav Immun*. 2007; 21:47–59. [PubMed: 16647243]
- Frank MG, Thompson BM, Watkins LR, Maier SF. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. *Brain Behav Immun*. 2012; 26:337–45. [PubMed: 22041296]
- Gregory NS, Gibson-Corley K, Frey-Law L, Sluka KA. Fatigue-enhanced hyperalgesia in response to muscle insult: induction and development occur in a sex-dependent manner. *Pain*. 2013; 154:2668–76. [PubMed: 23906552]
- Griebel G, Belzung C, Perrault G, Sanger DJ. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacol*. 2000; 148:164–70.
- Hadjimichael O, Kerns RD, Rizzo MA, Cutter G, Vollmer T. Persistent pain and uncomfortable sensations in persons with multiple sclerosis. *Pain*. 2007; 127:35–41. [PubMed: 16949751]
- Howe CL, LaFrance-Corey RG, Sundsbak RS, LaFrance SJ. Inflammatory monocytes damage the hippocampus during acute picornavirus infection of the brain. *J Neuroinflammation*. 2012a; 9:50–63. [PubMed: 22405261]
- Howe CL, LaFrance-Corey RG, Sundsbak RS, Sauer BM, LaFrance SJ, Buenz EJ, et al. Hippocampal protection in mice with an attenuated inflammatory monocyte response to acute CNS picornavirus infection. *Sci Rep*. 2012b; 2:545–57. [PubMed: 22848791]
- Johansen JP, Fields HL, Manning BH. The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. *Proc Natl Acad Sci U S A*. 2001; 98:8077–82. [PubMed: 11416168]
- Johnson JD, O'Connor KA, Deak T, Spencer RL, Watkins LR, Maier SF. Prior stressor exposure primes the HPA axis. *Psychoneuroendocr*. 2002a; 27:353–65.

- Johnson JD, O'Connor KA, Deak T, Stark M, Watkins LR, Maier SF. Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behav Immun*. 2002b; 16:461–76. [PubMed: 12096891]
- Johnson RR, Maldonado Bouchard S, Prentice TW, Bridegam P, Rassu F, Young CR, et al. Neonatal experience interacts with adult social stress to alter acute and chronic Theiler's virus infection. *Brain, Behavior, and Immunity*. 2014; 40:110–20.
- Johnson RR, Prentice TW, Bridegam P, Young CR, Steelman AJ, Welsh TH, et al. Social stress alters the severity and onset of the chronic phase of Theiler's virus infection. *J Neuroimmunol*. 2006; 175:39–51. [PubMed: 16631261]
- Johnson RR, Storts R, Welsh TH Jr, Welsh CJ, Meagher MW. Social stress alters the severity of acute Theiler's virus infection. *J Neuroimmunol*. 2004; 148:74–85. [PubMed: 14975588]
- Karelina K, Norman GJ, Zhang N, Morris JS, Peng H, DeVries AC. Social isolation alters neuroinflammatory response to stroke. *Proc Natl Acad Sci U S A*. 2009; 106:5895–900. [PubMed: 19307557]
- King T, Rao S, Vanderah T, Chen Q, Vardanyan A, Porreca F. Differential blockade of nerve injury-induced shift in weight bearing and thermal and tactile hypersensitivity by milnacipran. *J Pain*. 2006; 7:513–20. [PubMed: 16814690]
- King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, et al. Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci*. 2009; 12:1364–6. [PubMed: 19783992]
- King TE, Joynes RL, Meagher MW, Grau JW. Impact of shock on pain reactivity: II. Evidence for enhanced pain. *J Exp Psychol Anim Behav Process*. 1996; 22:265–78. [PubMed: 8691158]
- LaBuda CJ, Donahue R, Fuchs PN. Enhanced formalin nociceptive responses following L5 nerve ligation in the rat reveals neuropathy-induced inflammatory hyperalgesia. *Pain*. 2001; 94:59–63. [PubMed: 11576745]
- LaBuda CJ, Fuchs PN. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. *Exp Neurol*. 2000; 163:490–4. [PubMed: 10833324]
- Li J, Johansen C, Brønnum-Hansen H, Stenager E, Koch-Henriksen N, Olsen J. The risk of multiple sclerosis in bereaved parents: A nationwide cohort study in Denmark. *Neurol*. 2004; 62:726–9.
- Lipton HL, Melvold R. Genetic analysis of susceptibility to Theiler's virus-induced demyelinating disease in mice. *J Immunol*. 1984; 132:1821–5. [PubMed: 6699403]
- Lynch JL, Alley JF, Wellman L, Beitz AJ. Decreased spinal cord opioid receptor mRNA expression and antinociception in a Theiler's murine encephalomyelitis virus model of multiple sclerosis. *Brain Res*. 2008a; 1191:180–91. [PubMed: 18096140]
- Lynch JL, Gallus NJ, Ericson ME, Beitz AJ. Analysis of nociception, sex and peripheral nerve innervation in the TMEV animal model of multiple sclerosis. *Pain*. 2008b; 136:293–304. [PubMed: 17766043]
- McGavern DB, Zoecklein L, Drescher KM, Rodriguez M. Quantitative assessment of neurologic deficits in a chronic progressive murine model of CNS demyelination. *Exp Neurol*. 1999; 158:171–81. [PubMed: 10448429]
- McGavern DB, Zoecklein L, Sathornsumetee S, Rodriguez M. Assessment of hindlimb gait as a powerful indicator of axonal loss in a murine model of progressive CNS demyelination. *Brain Res*. 2000; 877:396–400. [PubMed: 10986359]
- McHugh TJ, Blum KI, Tsien JZ, Tonegawa S, Wilson MA. Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. *Cell*. 1996; 87:1339–49. [PubMed: 8980239]
- McLemore S, Crown ED, Meagher MW, Grau JW. Shock-induced hyperalgesia: II. Role of the dorsolateral periaqueductal gray. *Behav Neurosci*. 1999; 113:539–49. [PubMed: 10443780]
- Meagher MW, Ferguson AR, Crown ED, McLemore S, King TE, Sieve AN, et al. Shock-induced hyperalgesia: IV. Generality. *J Exp Psychol Anim Behav Process*. 2001; 27:219–38. [PubMed: 11497322]
- Meagher MW, Johnson RR, Young EE, Vichaya EG, Lunt S, Hardin EA, et al. Interleukin-6 as a mechanism for the adverse effects of social stress on acute Theiler's virus infection. *Brain Behav Immun*. 2007; 21:1083–95. [PubMed: 17591434]
- Mecha M, Carrillo-Salinas FJ, Mestre L, Feliu A, Guaza C. Viral models of multiple sclerosis: neurodegeneration and demyelination in mice infected with Theiler's virus. *Prog Neurobiol*. 2013; 101-102:46–64. [PubMed: 23201558]

- Mi W, Prentice TW, Young CR, Johnson RR, Sieve AN, Meagher MW, et al. Restraint stress decreases virus-induced pro-inflammatory cytokine expression during acute Theiler's virus infection. *J Neuroimmunol.* 2006; 178:59–61.
- Mohr DC, Hart SL, Julian L, Cox D, Pelletier D. Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. *BMJ.* 2004; 328:731. [PubMed: 15033880]
- Morgan MM, Heinricher MM, Fields HL. Inhibition and facilitation of different nocifensor reflexes by spatially remote noxious stimuli. *J Neurophysiol.* 1994; 72:1152–60. [PubMed: 7807200]
- Njenga MK, Asakura K, Hunter SF, Wettstein P, Pease LR, Rodriguez M. The immune system preferentially clears Theiler's virus from the gray matter of the central nervous system. *J Virol.* 1997; 71:8592–601. [PubMed: 9343217]
- Norman GJ, Karelina K, Zhang N, Walton JC, Morris JS, Devries AC. Stress and IL-1B contribute to the development of depressive-like behavior following peripheral nerve injury. *Mol Psychiatry.* 2010; 15:404–14. [PubMed: 19773812]
- Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD. Theiler's virus infection: a model for multiple sclerosis. *Clin Microbiol Rev.* 2004; 17:174–207. [PubMed: 14726460]
- Pardini M, Bergamino M, Bommarito G, Bonzano L, Luigi Mancardi G, Roccatagliata L. Structural correlates of subjective and objective memory performance in multiple sclerosis. *Hippocampus.* 2014; 24:436–45. [PubMed: 24375730]
- Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol.* 2014; 10:217–24. [PubMed: 24638131]
- Pollak Y, Ovadia H, Goshen I, Gurevich R, Monsa K, Avitsur R, et al. Behavioral aspects of experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2000; 104:31–6. [PubMed: 10683512]
- Qu C, King T, Okun A, Lai J, Fields HL, Porreca F. Lesion of the rostral anterior cingulate cortex eliminates the aversiveness of spontaneous neuropathic pain following partial or complete axotomy. *Pain.* 2011; 152:1641–8. [PubMed: 21474245]
- Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. *Neurol.* 1991; 41:685–91.
- Rodriguez M, Leibowitz JL, Lampert PW. Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. *Ann Neurol.* 1983; 13:426–33. [PubMed: 6340596]
- Roosendaal SD, Hulst HE, Vrenken H, Feenstra HE, Castelijns JA, Pouwels PJ, et al. Structural and functional hippocampal changes in multiple sclerosis patients with intact memory function. *Radiol.* 2010; 255:595–604.
- Ruet A, Deloire M, Hamel D, Ouallet JC, Petry K, Brochet B. Cognitive impairment, health-related quality of life and vocational status at early stages of multiple sclerosis: a 7-year longitudinal study. *J Neurol.* 2013; 260:776–84. [PubMed: 23081755]
- Sieve AN, Steelman AJ, Young CR, Storts R, Welsh TH, Welsh CJ, et al. Chronic restraint stress during early Theiler's virus infection exacerbates the subsequent demyelinating disease in SJL mice. *J Neuroimmunol.* 2004; 155:103–18. [PubMed: 15342201]
- Sieve AN, Steelman AJ, Young CR, Storts R, Welsh TH, Welsh CJ, et al. Sex-dependent effects of chronic restraint stress during early Theiler's virus infection on the subsequent demyelinating disease in CBA mice. *J Neuroimmunol.* 2006; 177:46–62. [PubMed: 16762424]
- Sorenson M, Janusek L, Mathews H. Psychological stress and cytokine production in multiple sclerosis: correlation with disease symptomatology. *Biol Res Nurs.* 2013; 15:226–33. [PubMed: 22084401]
- Truini A, Galeotti F, La Cesa S, Di Rezze S, Biasiotta A, Di Stefano G, et al. Mechanisms of pain in multiple sclerosis: a combined clinical and neurophysiological study. *Pain.* 2012; 153:2048–54. [PubMed: 22789132]
- Van'T Veer A, Carlezon WA Jr. Role of kappa-opioid receptors in stress and anxiety-related behavior. *Psychopharmacology.* 2013; 229:435–52. [PubMed: 23836029]
- Vichaya EG, Young EE, Frazier MA, Cook JL, Welsh CJ, Meagher MW. Social disruption induced priming of CNS inflammatory response to Theiler's virus is dependent upon stress induced IL-6 release. *J Neuroimmunol.* 2011; 239:44–52. [PubMed: 22000153]

- Vierck CJ, Hansson PT, Yeziarski RP. Clinical and pre-clinical pain assessment: are we measuring the same thing? *Pain*. 2008; 135:7–10. [PubMed: 18215466]
- Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: A review & analysis of alternative mechanisms. *Life Sci*. 1995; 57:1011–26. [PubMed: 7658909]
- Weil ZM, Norman GJ, Barker JM, Su AJ, Nelson RJ, DeVries AC. Social isolation potentiates cell death and inflammatory responses after global ischemia. *Mol Psychiatry*. 2008; 13:913–5. [PubMed: 18800053]
- Welsh CJ, Steelman AJ, Mi W, Young CR, Storts R, Welsh TH Jr. et al. Neuroimmune interactions in a model of multiple sclerosis. *Ann N Y Acad Sci*. 2009; 1153:209–19. [PubMed: 19236344]
- Welsh CJ, Tonks P, Nash AA, Blakemore WF. The effect of L3T4 T cell depletion on the pathogenesis of Theiler's murine encephalomyelitis virus infection in CBA mice. *J Gen Virol*. 1987; 68:1659–67. [PubMed: 2953860]
- Young CR, Schmitz HE, Atassi MZ. Antibodies with preselected specificities to protein regions evoked by immunization with free synthetic peptides: dose response to myoglobin antigenic sites reveal an optimum dose for each antigenic site. *Immunol Commun*. 1983; 12:419–28. [PubMed: 6195093]
- Young EE, Sieve AN, Vichaya EG, Carcoba LM, Young CR, Ambrus A, et al. Chronic restraint stress during early Theiler's virus infection exacerbates the subsequent demyelinating disease in SJL mice: II. CNS disease severity. *J Neuroimmunol*. 2010; 220:79–89. [PubMed: 20167380]
- Young EE, Vichaya EG, Reusser NM, Cook JL, Steelman AJ, Welsh CJ, et al. Chronic social stress impairs virus specific adaptive immunity during acute Theiler's virus infection. *J Neuroimmunol*. 2013; 254:19–27. [PubMed: 23021485]
- Ziehn MO, Avedisian AA, Tiwari-Woodruff S, Voskuhl RR. Hippocampal CA1 atrophy and synaptic loss during experimental autoimmune encephalomyelitis, EAE. *Lab Invest*. 2010; 90:774–86. [PubMed: 20157291]

Highlights

- Social stress before infection enhanced affective pain during the chronic phase.
- Stress and infection led to impaired memory, and infection led to more CA1 lesions.
- SJL mice showed infection-related motor deficits during the chronic phase of disease.

Figure 1.

Experimental timeline. Acute phase measures included sickness behavior, clinical scores, hind limb impairment, and open field activity. Chronic phase measures included clinical scores (ataxia and paresis), open field activity, footprint, rotarod, mechanical sensitivity, fear conditioning, novel object recognition, and immunological and histological analyses.

Figure 2.

Theiler's virus infection induced sickness behaviors in infected and stressed and infected mice. One-way ANOVAs using change from pre-infection baseline scores indicated that infected mice showed a greater loss of body weight (A) and a greater reduction in horizontal activity (D). The addition of stress prior to infection was associated with a significant decrease in vertical activity (C). No differences were seen in sucrose preference or mechanical sensitivity (B & E, respectively). Significant post hoc differences are indicated by asterisks for comparisons between groups at $p < .05$.

Figure 3.

Infection exacerbated acute phase behaviors in TMEV infected SJL mice. Repeated measures ANCOVAs suggest that infection increased clinical scores (A) and increased hind limb impairment (B) with infected animals showing varied vertical (C) and horizontal activity (D) over the acute phase. Significant post hoc differences are indicated by asterisks for comparisons between groups at $p < .05$.

Figure 4.

Effects of SDR on chronic phase behaviors in TMEV infected SJL mice. Repeated measures ANCOVAs suggest that infection led to a decrease in rotarod performance by day 161 pi (A) and increased ataxia and paresis scoring (B) while infection alone led to decreased hind stride length (C). Horizontal activity (D) decreased over time while no effect was seen in vertical activity (E). Insets signify main effects of group (B, C) or effect of infection for day 161 pi (A). Significant post hoc differences are indicated by asterisks for comparisons between groups and by pound signs (#) for comparisons between SDR infected and Non-SDR uninfected mice at $p < .05$.

Figure 5.

SDR enhanced nociception, pain-related behaviors, and memory consolidation. One-way ANOVAs suggest that SDR decreased mechanical threshold (A) and enhanced fear conditioning (B) in SDR infected mice. Using the recognition index, the SDR infected group had significantly less memory consolidation than the Non-SDR/Uninfected group (C). Significant post hoc differences are indicated by asterisks for comparisons between groups, by pound signs (#) for comparisons between SDR infected and Non-SDR uninfected mice, and by daggers (†) for comparisons between Non-SDR infected and the remaining two groups all at $p < .05$.

Figure 6.

Infection significantly increased the antibodies to TMEV virus throughout the disease course. Significant post hoc differences are indicated by asterisks for comparisons between groups at $p < .05$.

Figure 7.

Infection enhanced neuroinflammation in spinal cord and hippocampus. ANOVAs suggest that infection increased meningitis (A), microgliosis (B), and demyelination (C) in the spinal cord and infection increased demyelination in the CA1 region of the hippocampus (D). Non-SDR uninfected mice showed no lesions so their bar has been omitted. Significant post hoc differences are indicated by asterisks for comparisons between groups at $p < .05$.

Figure 8.

Representative sections of pyramidal neurons in the CA1 area of the hippocampus from each condition. No lesions were seen in the NonSDR uninfected group (A) while the NonSDR infected (B) and SDR infected (C) groups had significant focal loss of pyramidal neurons (arrows).