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Social disruption alters pain and cognition in an animal model of multiple sclerosis

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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 to prior Balb/cJ studies, stress failed to consistently alter behavioral and physiological indicators of disease course.

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). Similarly, cognitive impairments, such as memory deficits (Covey et al., 2011; Amato et al., 2001), are disruptive to daily living (Amato et al., 1995, 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, 2006a, 2006b, 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., 2004, 2006; 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, 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

Abbreviations: 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.

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chronic phases of TMEV infection in Balb/cJ mice (Johnson et al., 2004, 2006; Meagher et al., 2007; Vichaya et al., 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, 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., 1996, 2009; 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, 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 h 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, 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-day-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 month 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 the 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,