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Reduced sleep, stress responsivity, and female sex contribute to persistent inflammation-induced mechanical hypersensitivity in rats

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ABSTRACT

Studies in humans suggest that female sex, reduced sleep opportunities and biological stress responsivity increase risk for developing persistent pain conditions. To investigate the relative contribution of these three factors to persistent pain, we employed the Sciatic Inflammatory Neuritis (SIN) model of repeated left sciatic perineurial exposures to zymosan, an inflammatory stimulus, to determine their impact upon the development of persistent mechanical hypersensitivity. Following an initial moderate insult, a very low zymosan dose was infused daily for eight days to model a sub-threshold inflammatory perturbation to which only susceptible animals would manifest or maintain mechanical hypersensitivity. Using Sprague Dawley rats, maintaining wakefulness throughout the first one-half of the 12-h light phase resulted in a bilateral reduction in paw withdrawal thresholds (PWTs); zymosan infusion reduced ipsilateral PWTs in all animals and contralateral PWTs only in females. This sex difference was validated in Fischer 344, Lewis and Sprague Dawley rats, suggesting that females are the more susceptible phenotype for both local and centrally driven responses to repeated low-level inflammatory perturbations. Hypothalamic–pituitary–adrenal (HPA) axis hyporesponsive Lewis rats exhibited the most robust development of mechanical hypersensitivity and HPA axis hyperresponsive Fischer 344 rats matched the Lewis rats' mechanical hypersensitivity throughout the latter four days of the protocol. If HPA axis phenotype does indeed influence these findings, the more balanced responsivity of Sprague Dawley rats would seem to promote resilience in this paradigm. Taken together, these findings are consistent with what is known regarding persistent pain development in humans.

1. Introduction

Susceptibility factors for the development of persistent pain conditions are well known to include the three factors that were examined in this report, female sex, hypothalamic–pituitary–adrenal (HPA) axis stress responsivity, and sleep loss (Belfer et al., 2013; Clay et al., 2012; Holiday et al., 2010; Mogil, 2012; Smith et al., 2007a; Tang et al., 2012). Pre-clinical models of chronic neuropathic pain expand our knowledge of mechanisms of chronic neuropathic pain, but these models are limited in their usefulness for studying the development of persistent pain in several ways, including surgery-induced nerve trauma as the initiating pain event (Dowdall et al., 2005) and that a high proportion of animals exhibit relatively uniform and marked evoked mechanical sensitivity very soon after surgery (Mogil and Crager, 2004). In the human condition, inflammation is an aspect of virtually all neuropathic pain syndromes as initiator or consequence (Sommer, 2003); nerve trauma is not a consistent feature of persistent pain syndromes and the incidence and characteristics of neuropathic pain following surgery vary widely (Kehlet et al., 2006; Macrae, 2008).

To determine the contributions of sleep, stress responsivity and sex to the development of persistent pain, we employed the Sciatic Inflammatory Neuritis (SIN) model of perineurial inflammation-induced mechanical hypersensitivity in rats (Chacur et al., 2001; Milligan et al., 2004; Romero-Sandoval et al., 2005). The SIN model addresses several limitations of current preclinical neuropathic pain models, such as the separation of surgery from the initiating inflammatory insult and the ability to vary the dosing and timing of zymosan infusions via the exteriorized SIN catheter in the awake

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animal. Ipsilateral mechanical hypersensitivity is evident within 1 h of low dose zymosan infusion, 4 μg, which can be maintained with every other day infusion (Milligan et al., 2003). A ten-fold greater dose results in bilateral mechanical hypersensitivity (Chacur et al., 2001; Gazda et al., 2001; Romero-Sandoval et al., 2007; Romero-Sandoval and Eisenach, 2007; Romero-Sandoval et al., 2005). No studies employing the SIN model have reported including female rats.

We conducted two experiments in male and female rats using the SIN model to determine the risk of female sex, restricted sleep and stress responsivity on the development of zymosan-induced persistent mechanical hypersensitivity. We now report that our findings are consistent with studies in humans that female sex and reduced sleep opportunities confer risk for development of persistent pain. Our findings also suggest that both high and low HPA axis responsivity to moderate stress increase susceptibility to persistent pain.

2. Methods

2.1. Experimental design

Experiment 1: To determine the impact of sleep restriction on the development of persistent inflammation-induced mechanical hypersensitivity, male and female Sprague Dawley rats were assigned to one of four groups comprising a 2 × 2 experimental design: sleep restriction or not by zymosan infusion or not. Experiment 2: To determine whether HPA axis responsivity affects the development of persistent inflammation-induced mechanical hypersensitivity, male and female Sprague Dawley, Fischer 344 and Lewis rats were included. After undergoing tail tip blood withdrawal at baseline and following elevated plus maze (EPM) exposure to validate the respective high and low HPA axis stress responsivity of the inbred Fischer 344 and Lewis strains, animals were assigned to undergo zymosan infusion or not. Fig. 1 provides a depiction of the procedural time course for both experiments.

2.2. Animals

Male and female Sprague Dawley, Fischer 344 and Lewis rats from Harlan Laboratories (Indianapolis, IN) were bred in house after at least 3 weeks acclimatization to the vivarium or bred within two generations from in house bred animals. Two females were cohabited with a single male for 3 weeks. Pups were weaned at 21–22 days and housed 2–3 per cage by sex and strain. Age-matched mature offspring were entered into study at 4–7 months of age for all experiments. Animals had free access to food and water except only water was available for the 4 h before surgery. The vivarium was maintained at 22 ± 1 °C on a 12/12 light-dark cycle such that all testing, surgery and manipulations were accomplished during the dark phase. Given the necessity for extensive handling for these experiments, all animals were handled a minimum of three times per week for the month preceding surgery. All experimental protocols were approved by the Johns Hopkins University Institutional Animal and Care and Use Committee.

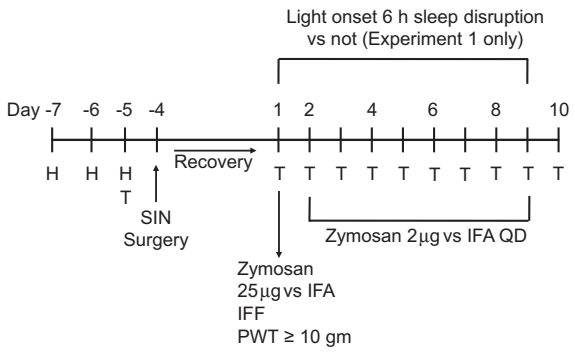

Fig. 1. Time course for pre and postoperative procedures for Experiments 1 and 2. H = habituation to von Frey Hair (vFH) testing environment; T = vFH testing; SIN = Sciatic Inflammatory Neuritis; IFF = if and only if; PWT = 50% paw withdrawal threshold; IFA = incomplete Freund's adjuvant; QD = every day.

2.3. Surgery

Animals underwent implantation of a chronic perisciatic catheter under isoflurane anesthesia (5% induction and 2–2.5% maintenance) following ampicillin prophylaxis (50 mg/kg subcutaneous [SC]), shave and betadine scrub. Briefly, after folding over and securing a gelfoam wrap to the left sciatic nerve at the mid-thigh level, the attached silastic catheter was tunneled subcutaneously and exteriorized into an aluminum covered protective plastic sleeve placed at the midline of the lower back. The procedures were the same as those described by Milligan et al. (Milligan et al., 2004) with four exceptions: (1) polyamide rather than silk suture was used to suture the gelfoam assembly to the quadriceps muscle to avoid possible inflammatory responses; (2) the silastic tubing was sutured to the protective sleeve to prevent slippage; (3) the length of the silastic tubing was adjusted to accommodate the sex- and strain-related differences in the distance from the mid-thigh to the lower back; and (4) 5–0 monofilament wire was used to suture the leg and back incisions rather than surgical clips. Upon completion of the procedure, all animals were injected with carprofen, 5 mg/kg SC, plus either morphine 10 mg/kg (males) in a slow release suspension (Page et al., 1993) or buprenorphine 0.03 mg/kg (females) SC. On the first postoperative day, an additional dose of carprofen was administered to all animals.

2.4. SIN catheter care and zymosan infusion

Beginning on postoperative day 5, animals were gently held and the end of the silastic tubing was exposed from the protective sleeve and gently suctioned using sterile technique each day. The zymosan (Sigma, St. Louis, MO) infusion dose was diluted in 50 μL incomplete Freund's adjuvant (IFA); IFA alone was used as the vehicle for Experiment 1. The silastic tubing was returned to the protective sleep upon completion of the infusion. The initial zymosan dose was administered only after the animal achieved a baseline 50% paw withdrawal threshold (PWT) of 10 gm using von Frey filaments.

The first zymosan dose was 25 μg, greater than 4 μg dose previously shown to result in unilateral mechanical sensitivity (Chacur et al., 2001; Gazda et al., 2001; Milligan et al., 2005b), and less than the 40 μg dose previously shown to result in bilateral mechanical sensitivity (Chacur et al., 2001; Gazda et al., 2001; Romero-Sandoval et al., 2005). This dose was selected to model an initial intermediate inflammatory insult, sufficient to initiate mechanical hypersensitivity of intermediate magnitude, 8 gm (Flatters and Bennett, 2004). This strategy models an initial pain insult that characterizes some chronic pains such as an injury, disease or surgery (Clay et al., 2012; Haroutiunian et al., 2013; Kehlet et al., 2006; Rosenbloom et al., 2013). Daily zymosan doses on days 2 through 9 were 2 μg, to model a sub-threshold inflammatory perturbation to which only susceptible animals would manifest or maintain mechanical hypersensitivity over time. Our goal with respect to the sub-threshold zymosan dose was to maximize the probability that we would have rats that would be resilient against the development of zymosan-induced mechanical hypersensitivity. By maximizing resilience, we could then discern whether specific biobehavioral influences, i.e., sex, sleep restriction, and