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Research Paper

Exploring acute-to-chronic neuropathic pain in rats after contusion spinal cord injury☆

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ABSTRACT

Spinal cord injury (SCI) causes chronic pain in 65% of individuals. Unfortunately, current pain management is inadequate for many SCI patients. Rodent models could help identify how SCI pain develops, explore new treatment strategies, and reveal whether acute post-SCI morphine worsens chronic pain. However, few studies explore or compare SCI-elicited neuropathic pain in rats. Here, we sought to determine how different clinically relevant contusion SCIs in male and female rats affect neuropathic pain, and whether acute morphine worsens later chronic SCI pain. First, female rats received sham surgery, or 150 kDyn or 200 kDyn midline T9 contusion SCI. These rats displayed modest mechanical allodynia and long-lasting thermal hyperalgesia. Next, a 150 kDyn (1 s dwell) midline contusion SCI was performed in male and female rats. Interestingly, males, but not females showed SCI-elicited mechanical allodynia; rats of both sexes had thermal hyperalgesia. In this model, acute morphine treatment had no significant effect on chronic neuropathic pain symptoms. Unilateral SCIs can also elicit neuropathic pain that could be exacerbated by morphine, so male rats received unilateral T13 contusion SCI (100 kDyn). These rats exhibited significant, transient mechanical allodynia, but not thermal hyperalgesia. Acute morphine did not exacerbate chronic pain. Our data show that specific rat contusion SCI models cause neuropathic pain. Further, chronic neuropathic pain elicited by these contusion SCIs was not amplified by our course of early post-trauma morphine. Using clinically relevant rat models of SCI could help identify novel pain management strategies.

1. Introduction

65–80% of individuals with spinal cord injury (SCI) experience chronic neuropathic pain (Siddall et al., 1999). Unfortunately, SCI-elicited pain is difficult to manage, and pain relief remains an important priority for individuals with SCI (Anderson, 2004; Collinger et al., 2013; Lo et al., 2016). Using rodent SCI models to study neuropathic pain mechanisms could help identify new pain modulatory targets. Several rat models of SCI-exacerbated evoked pain have been studied. Detloff et al. (2008) found that midline thoracic contusion SCI (moderate force) caused persistent mechanical allodynia in the hindpaw that correlated with increased inflammation in the lumbar spinal cord. Crown et al. (2008) showed that a T10 contusion SCI (150 kDyn force, 1 s dwell) caused mechanical allodynia by 35 days post-injury (dpi). Finally, unilateral cervical contusion SCI in rats caused persistent neuropathic pain the forepaw and hindpaw (Detloff et al., 2013; Putatunda et al., 2014). It is clear that midline and unilateral contusion SCI in rats causes some pain; however, the onset, duration, and relative magnitude of pain in SCI rat models has not been compiled in a single study.

Individuals with SCI are often treated with the opioid morphine to reduce post-SCI pain. Of individuals with post-SCI pain, 52–60% of patients received post-injury morphine (Cardenas and Jensen, 2006; Warms et al., 2002). According to patients, opioids are among the most helpful pain relievers (Cardenas and Jensen, 2006; Warms et al., 2002). Although morphine has beneficial acute analgesic effects that last hours (Cardenas and Jensen, 2006), opioid treatment also has adverse effects that include nausea, drowsiness, and constipation (Baastrup and Finnerup, 2008). In addition, studies from our group (Ellis et al., 2016; Grace et al., 2016) and others (Fletcher and Martinez, 2014; Laboureyras et al., 2014) suggest that opioids can have later paradoxical pain-enhancing effects. SCI rats treated with

Abbreviations: BBB, Basso-Beattie-Bresnahan (locomotor recovery scale); CNS, central nervous system; dpi, days post-injury; s.c., subcutaneous; SCI, spinal cord injury; SUDO, simplified up-down; T-, thoracic segment.

☆ **Summary**: Spinal cord injury pain models were compared. In female and male rats, midline spinal contusion caused persistent pain. Acute morphine did not worsen chronic pain.

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high-dose morphine show larger SCI lesions and impaired recovery of locomotor function (Hook et al., 2007; Hook et al., 2009; Hook et al., 2017). Using T13 dorsal root avulsion, our group found that subcutaneous morphine injections in rats for 7 days exacerbated later mechanical allodynia (Ellis et al., 2016). Extending these findings, here we test whether morphine also worsens later chronic pain in a rat model of clinically relevant contusion SCI.

Past studies assessed neuropathic pain in specific SCI models or timepoints. However, a systematic timecourse and comparison showing how different rat SCI models affect neuropathic pain has not been done. Here, we sought to establish an effective, clinically relevant rat model of SCI-elicited chronic neuropathic pain. Several thoracic contusion SCI models were tested, including moderate-to-severe midline contusion SCIs and a unilateral contusion SCI. A moderate force (150 kDyn) SCI with 1 s dwell was particularly effective at eliciting pain symptoms in male and female rats. Because morphine treatment in other nervous system trauma models can exacerbate later neuropathic pain, we hypothesized that acute post-injury morphine would worsen later chronic pain symptoms in our SCI models. Acute morphine treatment had expected analgesic effects and caused tolerance; however, later morphine-exacerbated pain was not observed.

2. Material and methods

2.1. Surgery and animal care

All housing, surgery, and postoperative care were approved by the University of Colorado Boulder Institutional Animal Care and Use Committee. All animals were fed standard chow and filtered tap water ad libitum and maintained on a 12:12 light/dark cycle. For all experiments, sham/SCI surgeries were interspersed throughout the day (during the light cycle). Female and male Sprague-Dawley rats (females: 200–250 g, males: 320–380 g; 2–3 months old; Harlan Laboratories) were anesthetized with isoflurane inhalation anesthesia, and both sham and SCI rats were treated with prophylactic antibiotics (gentamicin sulfate (Butler Schein), 1.25 mg s.c. in 0.25 mL sterile water). A partial T9 or T13 laminectomy was performed prior to SCI. The periosteum, but not the dura, was removed for all surgeries (this is the end of the surgery for sham rats). SCI rats were subjected to a contusion SCI using the Infinite Horizon device (Precision Systems and Instrumentation). Each experiment had distinct design and rat numbers: Experiment 1: 150 or 200 kDyn force, 0 s dwell with 1.5 mm impactor tip at midline T9 [females only: sham $n = 8$, 150 kDyn $n = 6$, 200 kDyn $n = 5$]; Experiment 2: 150 kDyn force, 1 s dwell with 1.5 mm impactor tip at midline T9 [females: sham-saline $n = 6$, sham-morphine $n = 6$, SCI-saline $n = 5$, SCI-morphine $n = 4$; males: sham-saline $n = 7$, sham-morphine $n = 6$, SCI-saline $n = 5$, SCI-morphine $n = 4$]; Experiment 3: 100 kDyn force, 1 s dwell with 1.0 mm-diameter impactor tip at left side T13 [males only: sham-saline $n = 6$; SCI-saline $n = 5$; SCI-morphine: $n = 6$].

Post-operative animal care included daily administration of gentamicin (both sham and SCI rats; 1 mL/d for 5 d), subcutaneous injection of Ringer's solution (5, 5, 4, 3, 2 mL on each of the first 5 days post-injury (dpi), respectively; for both sham and SCI rats) to prevent dehydration, and manual voiding of bladders twice daily (until recovery of bladder function at 2–3 weeks) (Gaudet et al., 2015). Animals were monitored daily for infection or other signs of suboptimal recovery. Rats were housed in pairs. Rats in all treatment groups were numbered randomly to ensure researchers were blind to group.

2.2. Morphine treatment

Rats were treated with morphine (or control saline) as previously described (Loram et al., 2012). (-)-Morphine sulfate (gift from NIDA Drug Repository; Research Triangle, NC, USA) was dissolved in sterile saline. Rats received 5 mg/kg s.c. morphine (or saline) 2× per day for 7 d (around the beginning and end of light phase), beginning on the first day post-surgery. To test for analgesic efficacy of morphine in Experiment 2, rat heat thresholds were tested prior to and 60 min after saline/morphine injection (on final day of morphine course, in the morning). To test for morphine analgesic efficacy and tolerance in Experiment 3, rat heat thresholds were tested prior to and 30, 60, 90, and 120 min after saline/morphine injection (after first injection and on final day of morphine course, in the morning).

2.3. Locomotor testing

Locomotor recovery was assessed using the Basso-Beattie Bresnahan (BBB) locomotor rating scale (Basso et al., 1995). A BBB score of zero represents no hindlimb movement; the highest BBB score of 21 represents typical coordinated and stable rat walking. Rating was performed by two researchers who were blind to treatment group. BBB scores were recorded at 1, 4, 7, 10, and 14 days post-injury (dpi), then weekly thereafter.

2.4. Neuropathic pain testing

Neuropathic pain assessment was completed as previously described (Ellis et al., 2016; Grace et al., 2016; McGraw et al., 2005). Rats were pre-acclimated to the von Frey and Hargreaves beakers for three sessions, and then had two pre-surgery tests. For each individual testing session, rats acclimated to their beaker for 40–60 min. Post-injury sensory testing occurred at least weekly post-injury. Unless otherwise noted, values for left and right hindpaws were averaged for each animal at each timepoint. The order of the rats in the beakers was randomized to ensure that the tester was blind to treatment group.

To assess mechanical sensory thresholds, the simplified up-down (SUDO) method (Bonin et al., 2014) of von Frey testing was used. This was used to limit rat stress and time out of home cage. Rats were placed on an elevated wire mesh, under a clear plastic beaker. After 40–60 min, the von Frey filaments (a logarithmic series of 10 calibrated Semmes-Weinstein monofilaments; Stoelting, Wood Dale, IL) were pressed against the center of the plantar surface of the rat hindpaw until they buckled and were held for a maximum of 5 s. The log stiffness of the hairs ranged from 3.61 (0.40 g) to 5.18 (15.14 g) filaments; the SUDO method started with filament 10 (4.31, 2.0 g). If the force of the filament elicited a definite paw withdrawal or notable flinch, a positive response was recorded. Whenever possible, von Frey testing was completed the day prior to Hargreaves testing to ensure accurate threshold measurements for both tests and to minimize rat stress.

To assess heat sensory thresholds (thermal hyperalgesia), the Hargreaves test was performed. Rats were placed on a glass platform under plastic beakers with an open top. An infrared source (intensity of 35) was placed under the center of their hindpaw and activated, then stopped once the rat moved their paw or flinched (clear nocifensive response to heat stimulus). Latency to response was automatically recorded. Testing on left and right hindpaws was alternated (three tests per timepoint), and 5–10 min elapsed between each test to limit sensitization. For morphine analgesia/tolerance, only two tests per paw per timepoint were used to limit hypersensitivity. Maximum response latency was set at 25 s; rats that did not respond at all over an individual test scored 25 s. This particularly occurred in some rats after acute morphine treatment.

2.5. Statistics

Data were analyzed using Student's *t*-test or non-parametric Mann-Whitney *U* test; or a one- or two-way ANOVA (repeated-measures, as appropriate), followed by Holm-Sidak post hoc test. Data were analyzed using SigmaPlot 12.0 (SPSS), and were considered significant when $p < 0.05$. All data are plotted as mean ± SEM.