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TITLE: A Novel Approach for Effectively Treating SCI Pain, Improving Opioid Efficacy, and Preventing Opioid-Induced Constipation: Key Role of Toll-Like Receptor 4 (TLR4)

PRINCIPAL INVESTIGATOR: Dr. Linda Watkins

CONTRACTING ORGANIZATION: Regents of the University of Colorado
Boulder, CO 80303

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14. ABSTRACT
Spinal cord injury (SCI) is a disabling and costly condition affecting wounded military personnel (1). SCI is also one of the leading causes of central neuropathic pain, and military personnel that sustain SCI in the field by improvised explosive devices (IEDs), grenades, gunshot wounds, etc. are at an even greater risk of developing chronic pain as well as emotional symptoms due to the polytraumatic nature of these injuries (2). Furthermore, central neuropathic pain in general is often intractable to treatment; current therapies including opioids only provide ~50% pain relief in 1 out of 2-3 people (3) and the therapies are even less effective in military blast SCI due to the complexity of the injury (4). This level of treatment efficacy is unacceptable for war fighters, military personnel, veterans, and citizens as a whole. SCI patients are almost universally treated with opioids as a first-line treatment, but recent evidence in the animal literature and recently in the clinical literature indicates that opioid administration after traumatic injury can have deleterious consequences. This proposal will test a clinically relevant therapeutic, (+)-naltrexone, that we predict will improve the efficacy of opioids for controlling SCI below-level pain while decreasing the negative consequences of opioid use. We predict that the mechanism by which (+)-naltrexone exerts at least some of its effects is via toll-like receptor 4. Thus our aim is to improve the quality of life for service-members, veterans, caretakers, and the general population.

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INTRODUCTION

Spinal cord injury (SCI) is a disabling and costly condition affecting wounded military personnel (Cross et al., 2011). SCI is also one of the leading causes of central neuropathic pain, and military personnel that sustain SCI in the field by improvised explosive devices (IEDs), grenades, gunshot wounds, etc. are at an even greater risk of developing chronic pain as well as emotional symptoms due to the polytraumatic nature of these injuries (Clark et al., 2009b). Furthermore, central neuropathic pain in general is often intractable to treatment; current therapies including opioids only provide ~50% pain relief in 1 out of 2-3 people (Beniczky et al., 2005) and the therapies are even less effective in military blast SCI due to the complexity of the injury (Clark et al., 2009a). This level of treatment efficacy is unacceptable for war fighters, military personnel, veterans, and citizens as a whole. SCI also causes bowel dysfunction ("neurogenic bowel dysfunction"), which is characterized by constipation and/or fecal incontinence (Stiens et al., 1997). SCI patients are almost universally treated with opioids as a first-line treatment, but recent evidence in the animal literature and recently in the clinical literature indicates that opioid administration after traumatic injury can have deleterious consequences (e.g., exacerbating chornic pain and constipation). This proposal will test a clinically relevant therapeutic, (+)-naltrexone, that we predict will improve the efficacy of opioids for controlling SCI below-level pain while decreasing the negative consequences of opioid use. We predict that the mechanism by which (+)-naltrexone exerts at least some of its effects is via toll-like receptor 4. Thus our aim is to improve the quality of life for service-members, veterans, caretakers, and the general population.

Keywords
Spinal cord injury, central neuropathic pain, rat, opioids, morphine, (+)-naltrexone, analgesia, allodynia, hyperalgesia, toll-like receptor 4

OVERALL PROJECT SUMMARY

Task 1. Obtain approval from the University of Colorado Institute Animal Care & Use Committee
(IACUC) for all animal work in the proposal. (Timeframe: 1-2 months prior to project start).
2a. Submit animal protocol covering all animal work at Boulder for IACUC meeting at least 2 months prior to anticipated start date (here, assumed to be September 1, 2013), hence July 2013 meeting or before. If revision required, submit for August 2013 meeting at latest.

Task 1 has been completed.

Milestone 1: Animal protocol is approved to allow funding to be received & to allow the project to start.

Milestone 1 has been completed.

Task 2. Purchase of, and corresponding training for operation of and maintenance of the Infinite Horizon impactor, for contusion spinal cord injury.
2a. Purchase of MASCIS (submission of sole source letter, ordering of equipment, and awaiting delivery).
2b. Team member will attend the training offered by W. M. Keck Center for Collaborative Neuroscience.

Task 2a Progress. Infinite Horizon spinal cord impactor (state-of-the-art in the SCI field) was purchased and assembled.
Task 2b Progress. Hired an expert in January 2015 for completing SCI surgeries. Dr. Andrew Gaudet, a Postdoctoral Research Associate, trained at The Ohio State University where he learned everything taught in the renowned Spinal Cord Injury Training Program. Since Gaudet already has training in SCI research, further training courses were not required. Since arriving, he assembled the device and has successfully run multiple rat and mouse contusion SCI experiments.

Task 3. Does co-administration of the TLR4 antagonist (+)-naltrexone with morphine prevent detrimental effects of morphine when this opioid is administered shortly after dorsal root avulsion, during the acute stages of injury?
3a. Spinal neuropathic avulsion pain (SNAP) surgery and testing (Hargreaves, motor function and constipation tests during the first week postsurgery with co-administration of morphine and (+)-naltrexone (vs. vehicles) starting 1 or 24 hr post surgery; von Frey and motor function testing weekly starting on day 8; Unblinding of data & data analysis) (Timeframe: September 2013 – February 2014)
3b. Histology: tissue processing (slicing staining, image collection, image analysis, and data analysis) (Timeframe: March 2014- May 2014)
     Task 3 Progress: Complete – see Milestone 3 discussion, below.

Milestone 2: Obtain the MASCIS impactor, as well as enhanced training using the MASCIS impactor, and knowledgeable on caring for, and maintaining of, the equipment.

MILESTONE 2 IS COMPLETE. Rather than purchasing the MASCIS impactor, the gold standard impactor for SCI research (Infinite Horizon) was purchased and assembled. It is now in

regular use.

Milestone 3: Determine if co-administration of morphine with (+)-naltrexone early following injury prevents the deleterious effects that occur when SCI is treated with morphine alone, if (+)-naltrexone enhances morphine analgesia, and if (+)-naltrexone also exerts positive outcomes in the absence of morphine. Determine if drug treatment alters the degree of injury in the spinal cord.

MILESTONE 3 IS COMPLETE. This quarter, **our group published an article in *Brain, Behavior, and Immunity*** showing that morphine worsened neuropathic pain symptoms after SNAP surgery. Worsened outcomes with morphine treatment correlated with increased spinal expression of inflammatory markers. Interestingly, the detrimental effects of morphine on SNAP-elicited neuropathic pain were ameliorated by treatment with (+)-naltrexone.

Task 4. Training on performing contusion spinal cord injury.
    4a. Team member to attend the Spinal Cord Injury Research Program Course at The Ohio State University.

Task 4a Complete. By hiring Gaudet, an experienced researcher from Ohio State with training on all aspects taught at the Spinal Cord Injury Training Program, the expense of sending a trainee to the course was avoided. Task 4a is complete.

MILESTONE 4 IS COMPLETE. Gaudet and his team of research assistants (Professional Research Assistant and undergraduates) completed contusion SCI surgery, behavior, and proposed experiments.

Task 5. Aim II: Does co-administration of the TLR4 antagonist (+)-naltrexone with morphine prevent detrimental effects of morphine when morphine is administered weeks after (6 weeks) dorsal root avulsion, after development of below-level neuropathic pain?

Milestone 5: Determine if co-administration of morphine with (+)-naltrexone after the development of established neuropathic pain reverses the deleterious effects that occur when SCI is treated with morphine alone, and if (+)-naltrexone enhances morphine analgesia, and if (+)-naltrexone also exerts positive outcomes in the absence of morphine. Determine if drug treatment alters the degree of injury in the spinal cord.
Milestone 5 Progress: Because the contusion SCI (which could have more broad clinical relevance), SCI-elicited neuropathic pain, and fecal collection strategies had to be developed (Task 6), these experiments took precedent over further studies using the SNAP model.

Task 6. Do robust phenomena from above generalize to the classic, widely employed SCI contusion model?
6b. Early co-administration of morphine and (+)-naltrexone: Contusion surgery and testing (only robust differences in above experiments will be pursued, and may be Hargreaves, motor function and/or constipation during the first week and co-administration of morphine and (+)-naltrexone

starting 1 or 24 hr post surgery; von Frey and motor testing weekly starting on day 8; Unblinding of data & data analysis) (Timeframe: January 2015 – September 2015)

6c. Late co-administration of morphine and (+)-naltrexone: Contusion surgery and testing (only robust differences in above experiments will be pursued, and may be Hargreaves, motor function and/or constipation during the week of co-administration of morphine and (+)-naltrexone at 6 weeks post surgery; von Frey and motor testing weekly starting upon completion of the week of drug administration; Unblinding of data & data analysis) (Timeframe: October 2015 – April 2016)

6d. Histology on 6b tissues: tissue processing (slicing staining, image collection, image analysis, and data analysis) (Timeframe: May 2016 – July 2016)

Milestone 6: Determine if deleterious consequences of morphine treatment of SNAP generalize to contusion spinal cord injury, and if these effects are prevented with co-administration of (+)-naltrexone. Also to define if (+)-naltrexone exerts positive outcomes in the absence of morphine.

Milestone 6 Progress: These experiments have been completed and are summarized in this report. SCI plus early post-injury morphine groups were studied in the context of contusion SCI pain and post-injury fecal dynamics. Morphine did lengthen whole-gut transit time after injury and had acute analgesic effects on post-SCI pain, but did not cause later severe constipation or post-treatment heightened neuropathic pain in this model. These results will be published in two papers within the next two months: one manuscript will discuss the SCI-elicited neuropathic pain models that we developed; the other will summarize our data suggesting that thoracic contusion SCI causes important changes to bowel function. We have ongoing analyses (no more rats) to better understand how SCI affects rat circadian activity/body temperature and cellular responses in the bowel.

The fact that SCI caused bowel dysfunction in our clinically-relevant rat model is particularly interesting for the field. To our knowledge, no one has ever studied post-SCI bowel dynamics in rat. Our study shows that T8 contusion SCI causes accelerated whole-gut transit time; i.e., incontinent-like symptoms that suggests the rats may have reduced nutrient absorption. Rats with SCI also showed disrupted time of fecal output, which could highlight disrupted circadian rhythms. This could have implications for recovery of function and quality/timing of sleep. Using these rodent models, researchers will be able to identify treatments that normalize fecal output dynamics, and to study circadian/sleep rhythms after SCI.

Milestone 7: Deliverable: Final report including study results and data analysis.
Milestone 7 complete.

<u>Task 6.</u> **Aim III: Do robust phenomena from above generalize to the classic, widely employed SCI contusion model?**
6b. Early co-administration of morphine and (+)-naltrexone: Contusion surgery and testing (only robust differences in above experiments will be pursued, and may be Hargreaves, motor function and/or constipation during the first week and co-administration of morphine and (+)-naltrexone starting 1 or 24 hr post surgery; von Frey and motor testing weekly starting on day 8; Unblinding of data & data analysis)

Task 6b. Progress.

1. EXPLORING SPINAL CORD INJURY-ELICITED NEUROPATHIC PAIN IN FEMALE AND MALE RATS

Midline T9 spinal cord injury in female rats elicits modest below-level pain symptoms
To determine how clinically relevant contusion SCI in a rodent model affects neuropathic pain symptoms, female rats were subjected to sham surgery, moderate 150 kDyn contusion SCI (no dwell time), or moderate-to-severe 200 kDyn contusion SCI (no dwell). Locomotor recovery (Basso, Beattie, and Bresnahan [BBB] scale for locomotor recovery) and pain symptoms (mechanical allodynia and thermal hyperalgesia) were studied over time.

Rats showed expected differences in locomotor recovery (BBB) score, with the rats subjected to moderate 150 kDyn SCI showing improved hindlimb movement compared to rats that received moderate-to-severe 200 kDyn SCI (overall group difference; also specific timepoints: 1 dpi, 7 dpi, 35 dpi, 42 dpi; $p < 0.05$) (Fig. 1.1, left panel). The average 150-kDyn BBB score at the final 42 dpi timepoint was 12, which corresponds to hindlimb plantar stepping with frequent coordination, whereas the average 200-kDyn BBB score at 42 dpi was approximately 11, corresponding to hindlimb plantar stepping with occasional coordination.
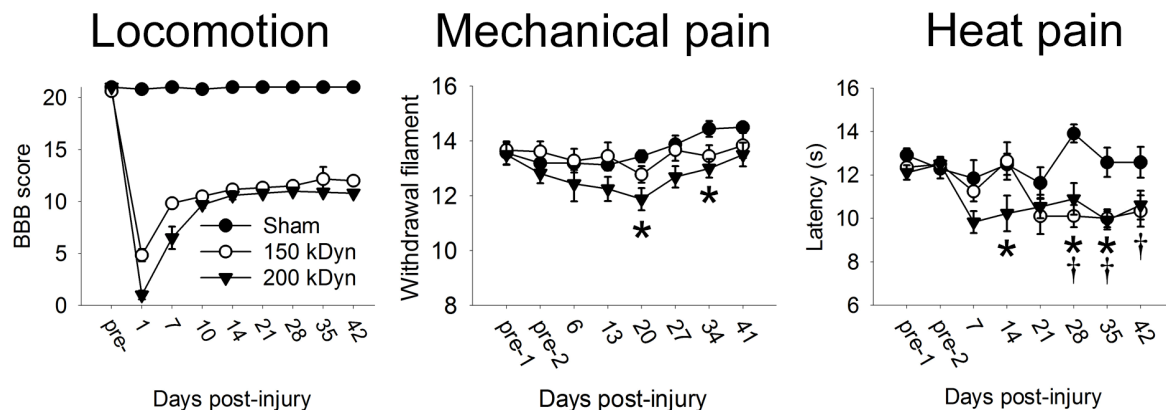

Figure 1.1. After moderate or moderate-severe midline contusion SCI (no dwell), female rats show expected post-SCI locomotor deficits (left panel) and modest neuropathic pain symptoms (middle panel: mechanical allodynia; right panel: thermal hyperalgesia). * indicates sham v. 200 kDyn, p < 0.05; ↑ indicates sham v. 150 kDyn, p < 0.05.

Rats with these SCIs showed modest post-injury neuropathic pain symptoms in the hindpaw. For mechanical allodynia (von Frey test), the more severe 200 kDyn group displayed significant hypersensitivity at 20 and 34 dpi (asterisks, compared to sham surgery rats at same timepoint; $p < 0.05$) (Fig. 1.1, middle panel). The 150 kDyn rats did not show significant SCI-induced

mechanical allodynia. The Hargreaves test was used to assess SCI-induced thermal hyperalgesia in the hindpaw. Both 150- and 200-kDyn SCI groups displayed heat hypersensitivity at several timepoints (200-kDyn at 14, 28, and 35 dpi, asterisks; and 150-kDyn at 28, 35, and 42 dpi, asterisks) (Fig. 1.1, right panel). Thus, the moderate and moderate-to-severe SCIs did elicit neuropathic pain symptoms; however, neuropathic pain was not consistent or particularly robust.

Midline T9 spinal cord contusion, with 1 second dwell, in female and male rats causes neuropathic pain

There are reports that neuropathic pain can be elicited by adding a 1 second "dwell time" to the impact. With dwell time, the contusion probe impacts the spinal cord to a known force; then, it remains in place compressing the spinal cord for 1 s prior to retracting. Thus, next we tested whether performing midline 150-kDyn contusion SCI at T9 with 1 s dwell elicited more robust pain symptoms. For locomotor recovery (BBB scale), females and males showed expected deficits and recovery patterns (Fig. 1.2a).

For neuropathic pain, rats displayed some neuropathic pain symptoms with the dwell time – particularly relating to thermal hyperalgesia. For mechanical allodynia (Fig. 1. 2b), female rats did not show significant neuropathic pain (compared to sham rats at same timepoint), whereas male rats had significant mechanical allodynia beginning at 14 dpi and persisting through 42 dpi ($p < 0.05$). For thermal hyperalgesia (Fig. 1.2c), female rats had significant neuropathic pain (compared to sham rats at same timepoint) at 14, 35, and 42 dpi ($p < 0.05$). Male rats showed significant thermal hyperalgesia at 14, 21, 28, 35, and 42 dpi ($p < 0.05$). Thus, we found that this SCI model elicited significant thermal hyperalgesia in females and males, and mechanical allodynia specifically in males.
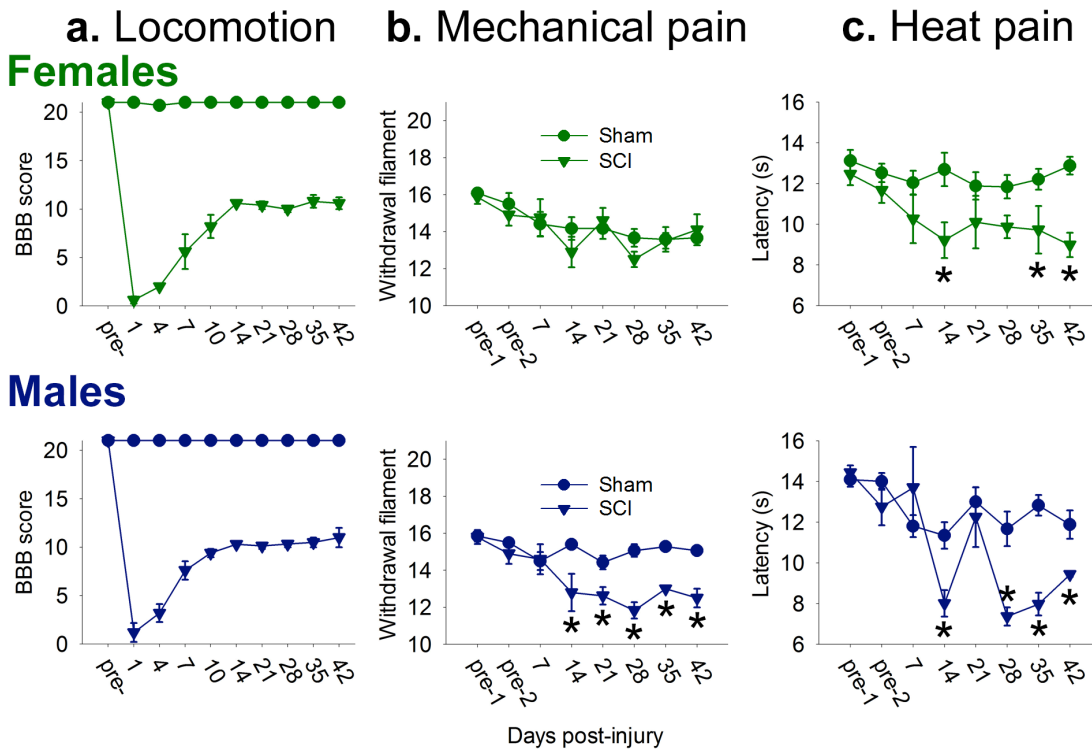
Figure 1.2. Midline 150-kDyn SCI with 1 s dwell causes locomotor deficits and neuropathic pain in female (top panels) and male (bottom panels) rats. (a) Female and male rats with SCI show similar locomotor deficits and recovery after SCI. (b) Male, but not female SCI rats exhibit significant mechanical allodynia. (c) Both female and male SCI rats display significant thermal hyperalgesia at several post-surgery timepoints. * indicates $p < 0.05$ vs. sham at same timepoint.

After midline T9 spinal cord injury, acute morphine did not exacerbate neuropathic pain
Based on previous work by our lab and others, we expected that an acute course of morphine would slow locomotor recovery and exacerbate SCI-induced neuropathic pain symptoms. To examine this, rats were subjected to sham or SCI surgery (as in Fig. 1.2 – same animals; including all groups [saline/morphine] here to enable effective explanation) then were injected with control saline solution or morphine (5 mg/kg, 2x per day for 7 d beginning at 1 dpi) (Fig. 1.3). Our lab previously showed that this treatment worsens later neuropathic pain in peripheral pain models (chronic constriction injury) and in a dorsal root avulsion CNS injury model. The morphine treatment had a negative effect on the rats' appearance and condition; SCI rats treated with morphine had more piloerection (raised fur) and more squinted eyes, suggesting their recovery of health took longer.
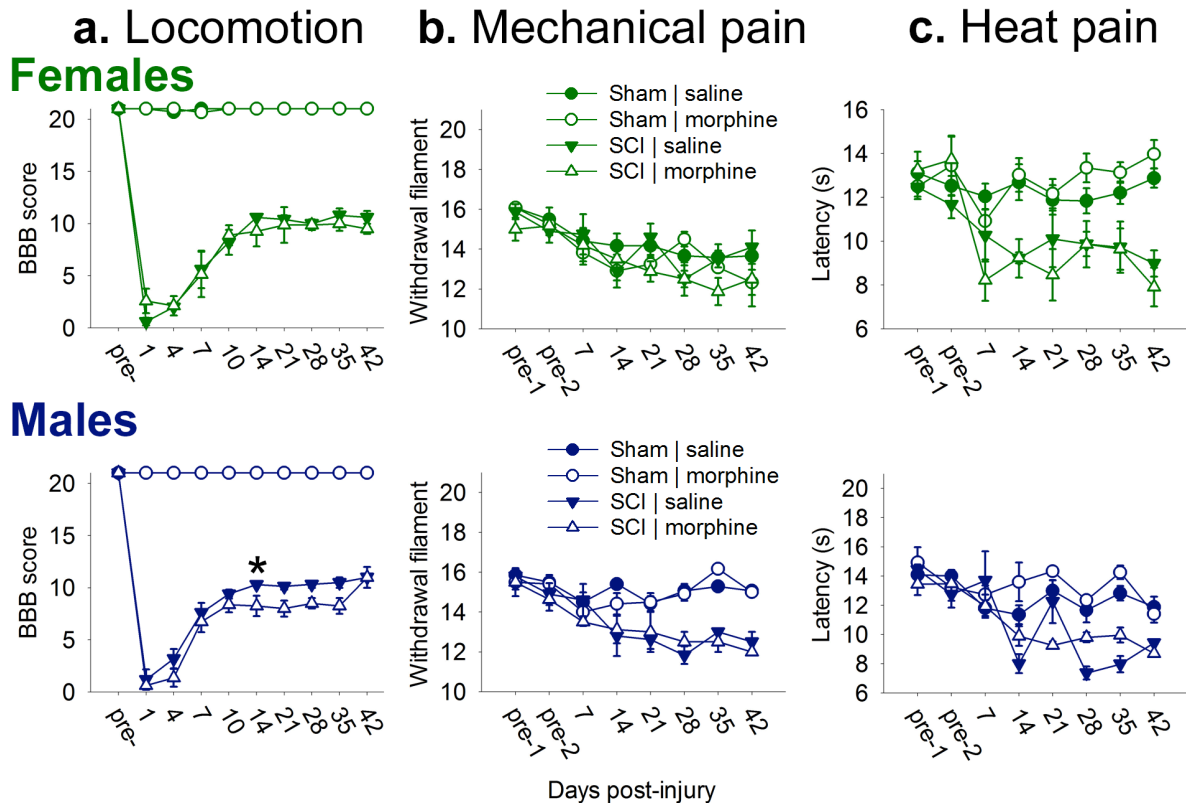
Figure 1.3. Morphine did not exacerbate neuropathic pain after midline contusion SCI in female and male rats. (a) Acute treatment with morphine (5 mg/kg 2x per d for 7 d post-surgery, beginning at 1 dpi) had no significant effect on post-SCI locomotor recovery in females, and a modest detrimental effect on BBB score in males. (b) SCI-elicited mechanical allodynia was not significantly worsened by morphine. (c) SCI-induced heat pain was not exacerbated by acute morphine treatment. * indicates SCI-saline vs. SCI-morphine, $p < 0.05$.
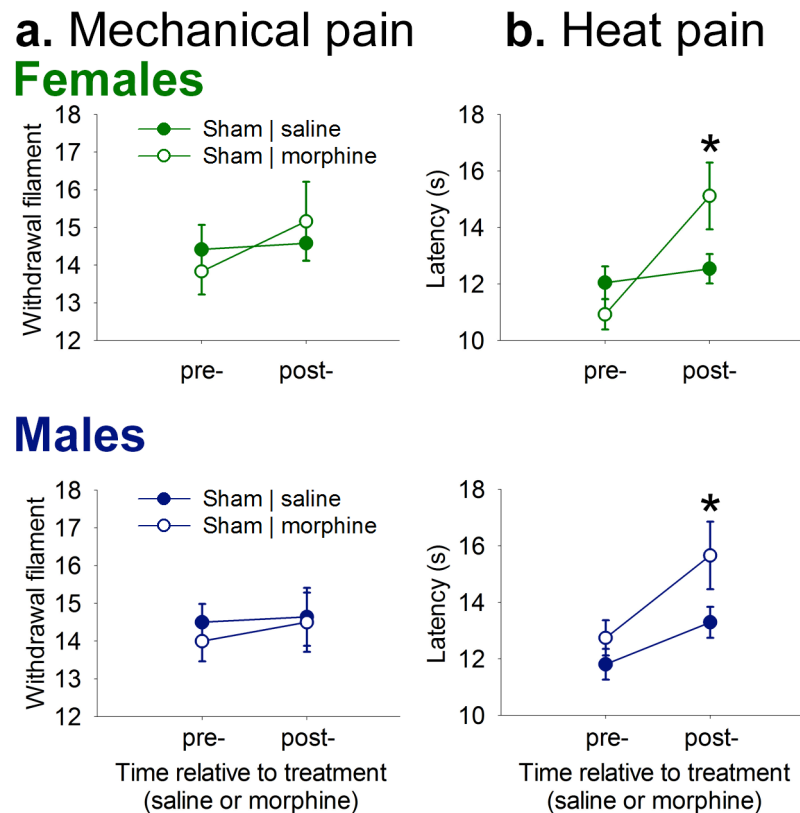
Acute morphine had little effect on later locomotor recovery and neuropathic pain symptoms. For locomotor recovery (Fig. 1.3a), SCI females on morphine did not show significant differences in BBB scores over the timecourse. Males with SCI on morphine did perform significantly worse, though this occurred only at 14 dpi. One challenge with SCI males is that their bladders are prone to bursting; this afflicted several males and they had to be euthanized. Thus, although we present data for the males to 42 dpi here, the data with sufficient power for statistical analyses are mainly between 1-21 dpi.

Mechanical allodynia and thermal hyperalgesia were assessed (Fig. 1.3b,c). We predicted that the acute morphine course would worsen later (i.e., chronic) neuropathic pain. Here, there were no significant differences between saline- and morphine-treated rats, both in females and males.

Morphine had expected acute analgesic effects

Since morphine had no significant effect on later chronic neuropathic pain, we sought to confirm whether the morphine was effective. Rats were tested for mechanical (Fig. 1.4a) and thermal (Fig. 1. 4b) thresholds prior to, and 40-60 min after treatment with morphine or saline (only shams are presented for clarity). There was a significant effect of morphine on thermal thresholds. Indeed, both female and male rats treated with morphine showed significantly lengthened latencies to respond to thermal stimuli. SCI rats treated with morphine had delayed responses compared to saline-treated rats, and these latencies were also significantly longer than

Figure 1.4. Morphine had expected analgesic effects on heat thresholds. To test morphine efficacy, female and

male rat thresholds were tested on the 7th day of morphine treatment prior to injection and at ~60 min post-injection. (a) At this time, morphine had little effect on mechanical thresholds. (b) Acute post-morphine heat thresholds were significantly extended in female and male rats, highlighting its efficacy. In future studies, testing morphine efficacy at earlier times and testing morphine tolerance were included. * indicates morphine- vs. saline-treated, $p < 0.05$.

those of these rats prior to morphine treatment. There was no significant effect of morphine treatment on mechanical thresholds, and there are several possible explanations. First, they were tested at 40 min post-morphine; perhaps a timecourse could have identified analgesia. Second, the rats were only tested on the second-last day of morphine delivery; morphine tolerance had likely already set in. Therefore, in our next experiment we improved design to account for these possibilities.

These results suggest that morphine was having its intended effects, and that perhaps morphine does not worsen chronic SCI pain in this injury model.

Unilateral thoracic spinal cord injury causes neuropathic pain that is not altered by morphine or (+)-naltrexone

It is possible that morphine-exacerbated chronic neuropathic pain may be observed in other SCI models. Another contusion SCI model known to cause neuropathic pain is a unilateral contusion SCI. Here, we adapted the unilateral injury to the thoracic level, which would enable comparing results to our previous experiments and testing below-level pain in the male rat hindpaw. Thus, a 100-kDyn contusion SCI (with a 1.0-mm diameter impactor tip) was performed on the left side of the T9 spinal cord. Sham surgery was included; this consisted of a T9 laminectomy without spinal cord impact. In addition, we tested the hypotheses that acute morphine would worsen locomotor recovery and chronic neuropathic pain, and that acute (+)-naltrexone could benefit functional recovery and pain symptoms.

Rats with unilateral SCI exhibited hindpaw locomotor deficits that were more severe on the side ipsilateral to injury. The ipsilateral hindpaw of saline-treated SCI rats had an average BBB score of 2.8 at 1 dpi, and recovered to 13.25 by the final 42 dpi timepoint (Fig. 1.5a). The hindpaw contralateral to injury in saline-treated SCI rats had an average BBB score of 11 at 1 dpi, and recovered to 15.5 by the final 42 dpi timepoint. Morphine or (+)-naltrexone had no significant effect on recovery of function. Thus, our data show that rats have expected worsened below-level motor function on the side ipsilateral to injury, and that morphine or (+)-naltrexone did not modify locomotor recovery.
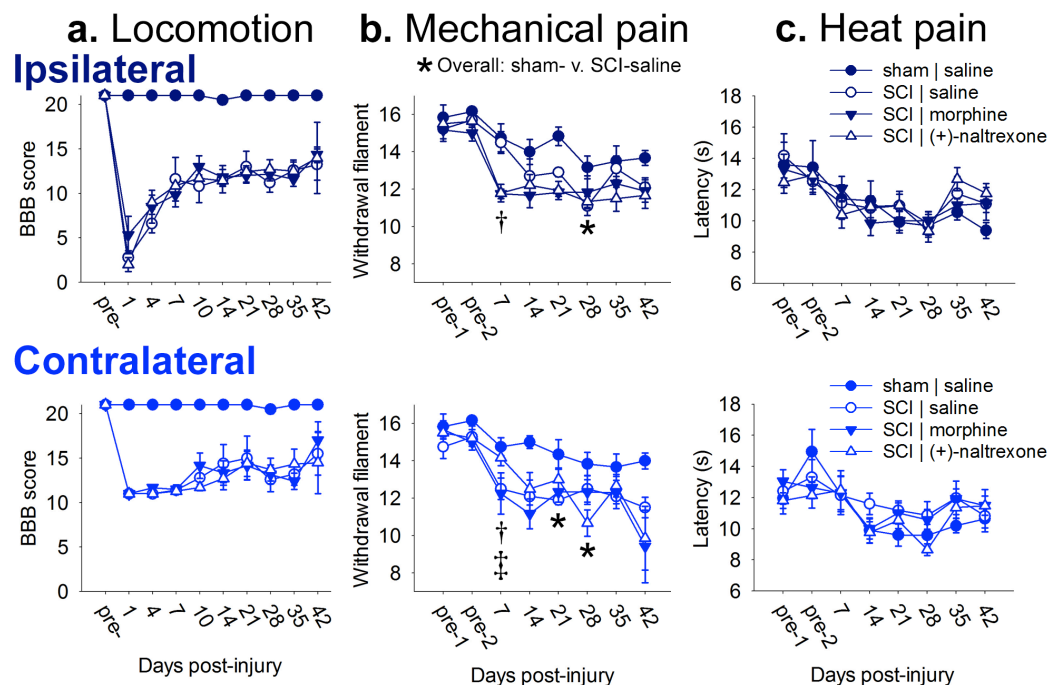

Figure 1.5. Unilateral SCI in male rats caused locomotor deficits and mechanical allodynia. (a) After unilateral SCI, locomotor deficits are worse on the side ipsilateral to injury (* sham-saline vs. SCI-saline). Morphine or (+)-naltrexone did not affect recovery. (b) Unilateral SCI caused mechanical neuropathic pain on both ipsilateral and contralateral hindpaws (*). Morphine († vs. SCI-saline) and (+)-naltrexone (‡ vs. SCI-saline) worsened post-SCI pain at 7 dpi. (c) Unilateral SCI did not elicit significant thermal hyperalgesia on either hindpaw; morphine or (+)-naltrexone did not alter responses in the Hargreaves test.

Unilateral SCI caused mechanical allodynia in both ipsilateral and contralateral hindpaws. On the ipsilateral hindpaw, saline-treated rats with SCI exhibited significant ipsilateral mechanical neuropathic pain (compared to sham rats; overall group difference, $p < 0.05$). Saline-SCI rats also showed significant ipsilateral mechanical pain specifically at 28 dpi (vs. sham-saline rats; $p < 0.05$). Unilateral SCI-induced pain appeared to be more robust on the contralateral side (vs. sham-saline rats; $p < 0.05$). On the contralateral hindpaw, SCI-saline rats displayed significant mechanical allodynia at 21 and 28 dpi.

Morphine and (+)-naltrexone modestly exacerbated unilateral SCI-induced pain. On the side ipsilateral to SCI, morphine treatment worsened SCI mechanical pain at 7 dpi ($p < 0.05$). It was predicted that (+)-naltrexone would improve SCI pain symptoms; however, here (+)-naltrexone actually worsened ipsilateral neuropathic pain (group effect vs. SCI-saline, $p < 0.05$). Similarly, on the contralateral hindpaw, both morphine and (+)-naltrexone worsened SCI-induced neuropathic pain (vs. SCI-saline; overall group effect and specifically at 7 dpi). Thermal hyperalgesia was also examined in these unilateral SCI rats. Both ipsilateral and contralateral to SCI, there was no significant hypersensitivity observed (SCI-saline vs. sham-saline rats; $p > 0.05$). In addition, there was no significant effect of morphine or (+)-naltrexone on heat thresholds over the timecourse (vs. SCI-saline; $p > 0.05$).

In the unilateral SCI model, morphine had expected analgesic effects and tolerance properties
To confirm that morphine was having its known effects, a more thorough examination of analgesic and tolerant properties was performed using the Hargreaves test. Hindpaw responses were averaged. At 1 dpi – immediately after the first morphine injections, when it is expected to have its strongest analgesic potency – morphine increased heat thresholds (analgesia) as soon as 30 min post-injection and maintained strong analgesic effects through 90 min. At 120 min, the analgesic effects of the single morphine dose began to decay. To test for tolerance, thresholds were tested again at 7 dpi (after 6 d of twice-daily saline/morphine/(+)-naltrexone injections). Thresholds were tested prior to and 60 min post-injection – a time of maximal morphine efficacy. Comparing the percent change from baseline at 1 dpi and 7 dpi, morphine is the only treatment that had significant analgesic effects at 1 dpi ($p < 0.05$). At 7 dpi, these analgesic effects were less robust (~50% less analgesia at 7 dpi compared to 1 dpi; % change not significantly different from saline- or naltrexone-treated groups). Therefore, morphine had analgesic properties, and the 7 d course of morphine led to expected morphine tolerance in the unilateral SCI model.
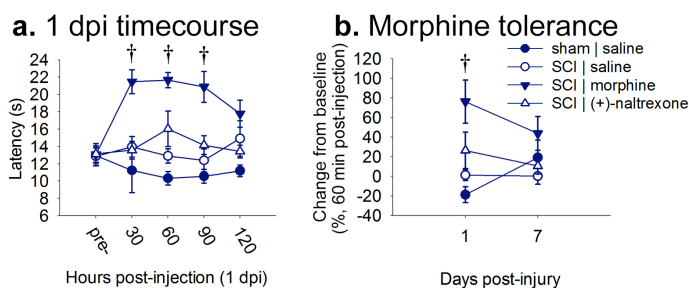

a. 1 dpi timecourse

b. Morphine tolerance

Figure 1.6. After unilateral SCI, morphine had expected analgesic effects and tolerance properties. (a) Male rats treated with their first injection of morphine show lengthened latency to response to heat stimuli. (b) Rats treated with morphine for 6 d exhibit analgesic tolerance. Heat thresholds were tested prior to and 60 min post-

injection; morphine had significant analgesic effects at 1, but not at 7 dpi. † indicates SCI-saline vs. SCI-morphine, $p < 0.05$.

2. SPINAL CORD INJURY IN RATS DISRUPTS CIRCADIAN RHYTHMS AND BOWEL FUNCTION

Spinal cord injury causes transient reduction in body mass and altered food intake

To establish how SCI affects body mass, sham and SCI rats were weighed at various times post-surgery (Fig. 2.1). Both female and male rats had reduced body mass by 4 dpi, that recovered with time post-injury. Female rats with SCI had body masses that were not significantly different from shams at 21 dpi, whereas male rats recovered to sham levels at 28 dpi.

Body mass changes after SCI coincided with altered food intake. Female rats with SCI increased daily food intake over sham rats at 7 and 14 dpi. Male rats showed reduced food intake at 2 dpi, but increased food intake over sham rats at 7 and 14 dpi. These results show that SCI causes transient weight loss, and that increased post-SCI food intake could eventually help recover to typical body mass.
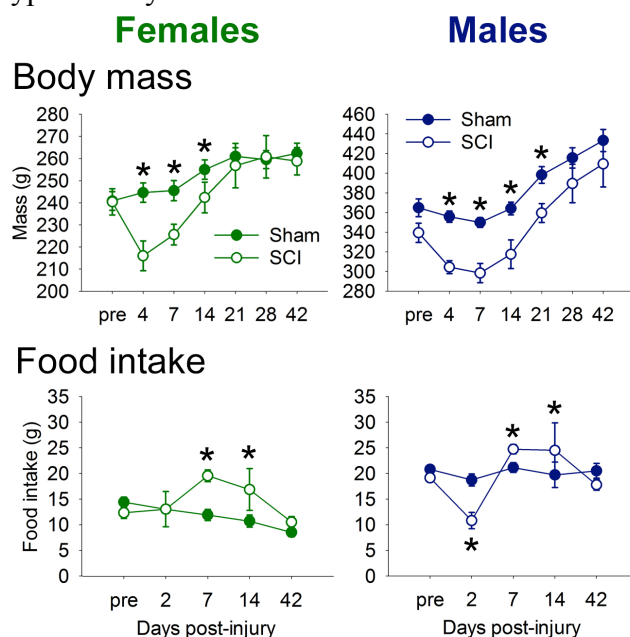

Figure 2.1. SCI in females and males transiently reduces body mass (top panels), which is followed by a compensatory increase in food intake (bottom panels).

Spinal cord injury reduces whole-gut transit time

To determine how SCI affects whole-gut transit time – which could impact nutrient/fluid absorption and overall health – rats were gavaged with Evan's blue dye and the time taken to pass blue fecal pellets was assessed (Fig. 2.2). Rats prior to SCI surgery had an average whole-gut transit time of 9.8 ± 0.7 h (females) and 9.7 ± 0.7 h (males). Interestingly, T9 contusion SCI accelerated whole gut transit time: At 7 dpi, both female and male SCI rats had shorter whole gut transit times compared to sham rats (female sham: 10 ± 1 h; female SCI: 7.2 ± 0.8 h; $U(27) = 38.5$, $p < 0.005$) (male sham: 7.8 ± 0.5 h; male SCI: 7.1 ± 0.4 h; $U(26) = 33.0$, $p < 0.005$). This SCI-expedited whole gut transit was maintained at 14 dpi (female sham: 10 ± 1 h; female SCI: 7.2 ± 0.8 h; $U(9) = 2.00$, $p < 0.05$) (male sham: 7.1 ± 0.7 h; male SCI: 4.5 ± 0.4 h; $U(15) = 0.0$, $p < 0.005$). At 42 dpi, only male rats had a significantly shorter whole-gut transit time (male sham: 11.6 ± 0.8 h; male SCI: 8.3 ± 0.8 h; $t(15) = 2.94$, $p < 0.01$) (though there was a similar but non-significant pattern in females). These differences in whole-gut transit were not accompanied by

measureable differences in food or water intake, or fecal water content. Thus, thoracic contusion SCI in female and male rats expedited whole-gut transit time.

Spinal cord injury increases fecal pellet and fecal mass output
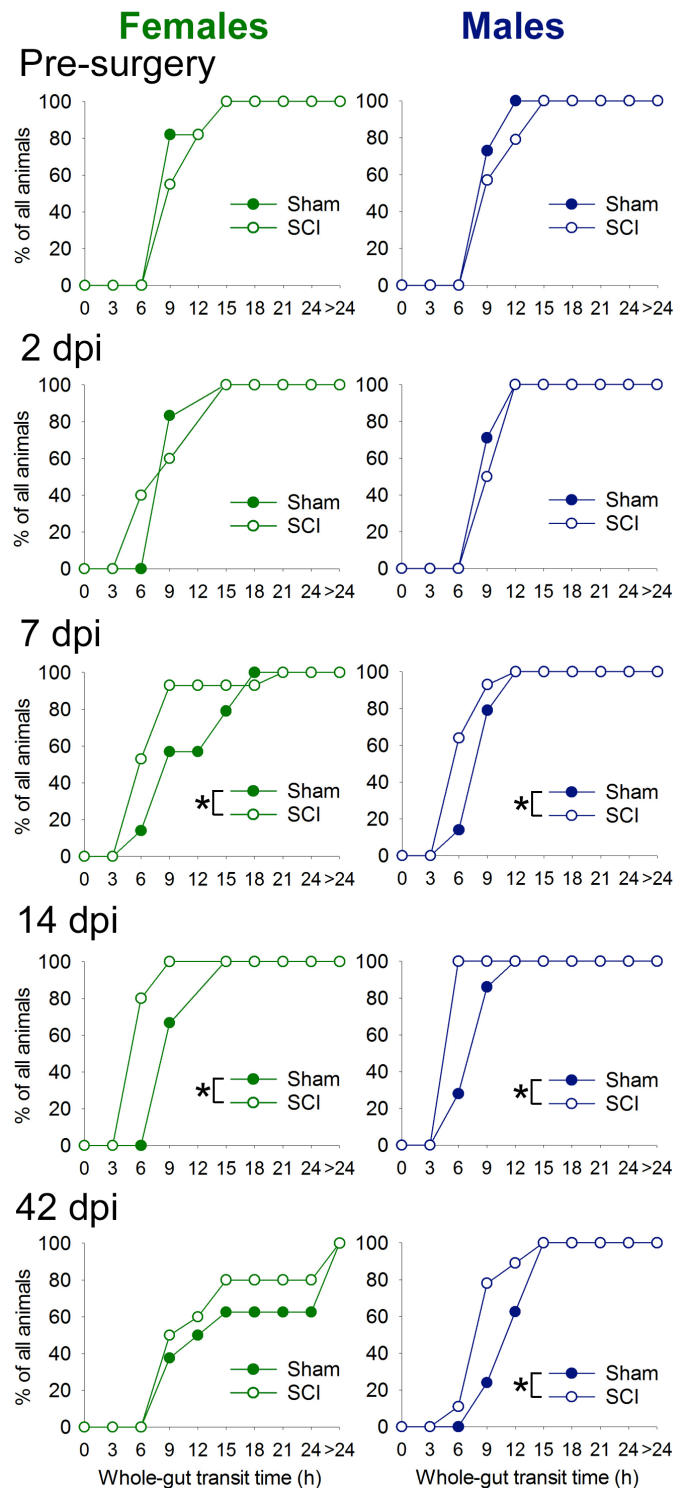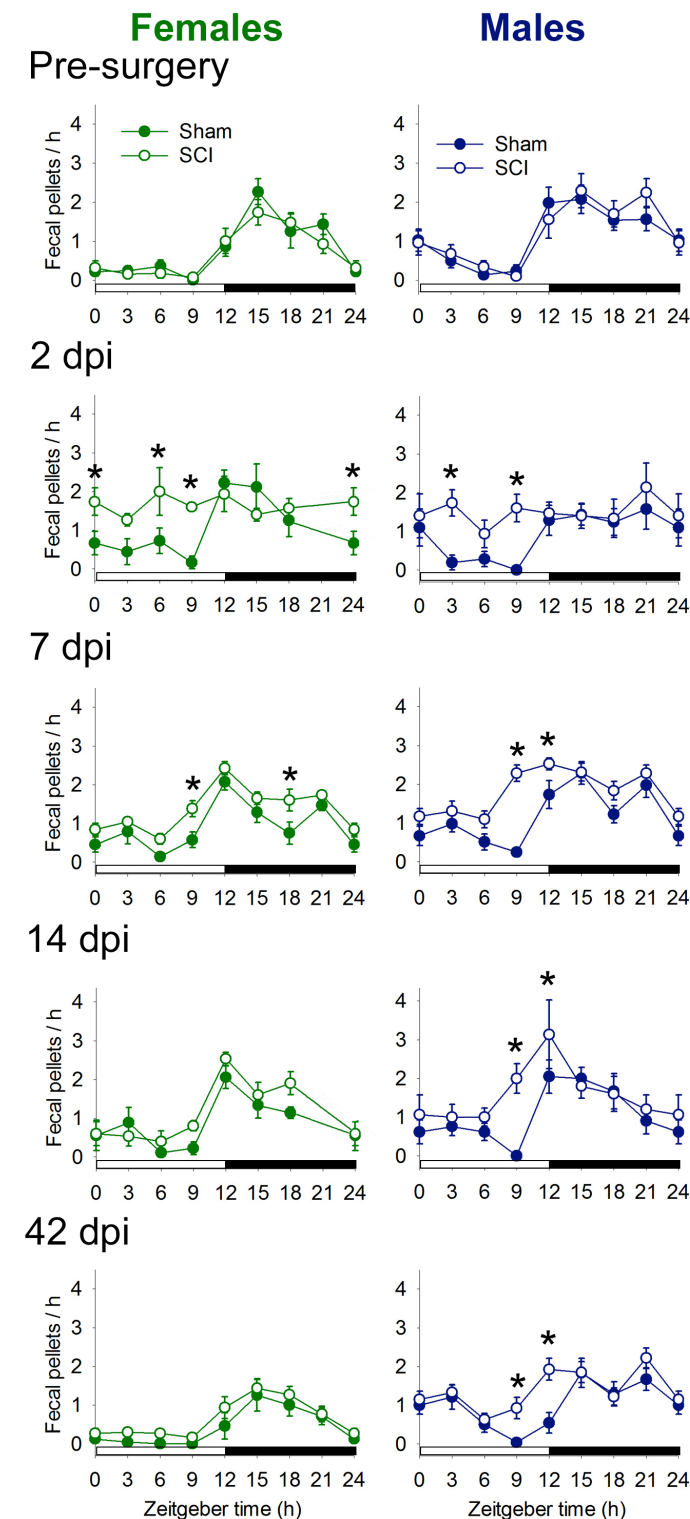

Rats with SCI could also have altered overall fecal output. Fecal output (pellet number and mass) was measured prior to surgery, and at 2, 7, 14, and 42 dpi (Fig. 2). Prior to surgery, SCI female rats released 18 ± 3 pellets over 24 h; male rats released 27 ± 3 pellets over 24 h. Both female and male rats with SCI increased pellet production compared to sham rats (Fig. 2.3, upper panels) (main effect of treatment: female: $F(1,27)=11.61$, $p < 0.005$; male: $F(1,27)=10.53$, $p < 0.005$). Pellet release by SCI female and male rats increased at 2 dpi (female sham: 26 ± 4 pellets; female SCI: 29 ± 6 pellets; $p < 0.01$) (male sham: 17 ± 3 pellets; male SCI: 32 ± 6 pellets; $p < 0.05$) and at 7 dpi (female sham: 24 ± 2 pellets; female SCI: 34 ± 2 pellets; $p < 0.001$) (male sham: 27 ± 2 pellets; male SCI: 40 ± 3 pellets; $p = 0.001$). At 14 dpi, female but not male rats with SCI had significantly increased fecal pellet production (female sham: 22 ± 2 pellets; female SCI: 31 ± 3 pellets; $p < 0.05$) (male sham: 19 ± 5 pellets; male SCI: 30 ± 7 pellets; $p = 0.059$). Neither female nor male rats with SCI had significantly altered pellet production at 42 dpi.

Fig. 2.2. SCI in female and male rats accelerates whole-gut transit time. Rats were gavaged with Evan's blue dye; then, the time to passing blue fecal pellets was recorded. Cumulative distributions are shown. Moderate T9 contusion SCI expedited whole-gut transit time in female and male rats beginning at 7 dpi and lasting to 14 dpi in females (though similar pattern observed at the chronic 42 dpi time) and 42 dpi in males. * indicates group difference at respective timepoints.

SCI in both sexes also increased daily fecal mass production (Fig. 2.3, lower panels) (main effect of treatment: female: $F_{(1,27)}=8.46$, $p < 0.01$; male: $F_{(1,27)}= 4.54$, $p < 0.05$). Prior to surgery, female rats in the SCI group produced 3.7 ± 0.5 g and males produced 7.0 ± 0.4 g fecal mass over 24 h. Females showed SCI-induced increases in fecal mass at 7 dpi (sham: 4.9 ± 0.4 g; SCI: 7.6 ± 0.4 g; $p < 0.001$) and at 14 dpi (sham: 4.8 ± 0.5 g; SCI: 7.8 ± 0.6 g; $p < 0.005$). Males with SCI increased fecal mass production at 14 dpi (sham: 7 ± 1 g; SCI: 12 ± 2 g; $p < 0.001$). Thus, SCI transiently increases overall production of fecal pellets and mass.

Spinal cord injury disrupts typical circadian rhythm of fecal production
To establish whether SCI altered timing of fecal output, fecal pellets were collected every 3 h over a 24 h period prior to and at several times after surgery. Fecal pellet number (Fig. 2.4) and mass (Fig. 2.5) were measured throughout the light phase (inactive; ZT0-12) and dark phase (active; ZT12-24).

Prior to surgery, fecal pellet output (Fig. 2.4) from female and male rats showed the expect daily pattern: Fecal pellet output was increased at the beginning of the active (dark) phase. In sham rats, this pattern was maintained after surgery; however, female and male rats with SCI showed disrupted rhythms of fecal output. Circadian rhythms of fecal pellet

Fig. 2.3. Circadian timing of fecal pellet output is altered after SCI in female and male rats. Fecal pellet number was recorded every 3 h for 24 h prior to surgery and at several post-surgery timepoints. SCI caused robust circadian disruption of fecal pellet output at 2 dpi (unusually high output during the inactive [light] phase; ZT 0-12). Fecal pellet output rhythms remained disrupted at 7 dpi in females and males. In males, disrupted rhythms persisted at 14 and 42 dpi.

output were disrupted in female and male rats at 2 dpi

and 7 dpi. Males, but not females, also showed persistent disruption of fecal output rhythms at 14 dpi and at a chronic timepoint, 42 dpi.

Rhythms in fecal mass output (Fig. 2.5) were similarly disrupted by SCI. Females and males showed altered rhythms in fecal mass output at 2, 7, and 14 dpi. Males, but not females, showed persistent disruption at 42 dpi.
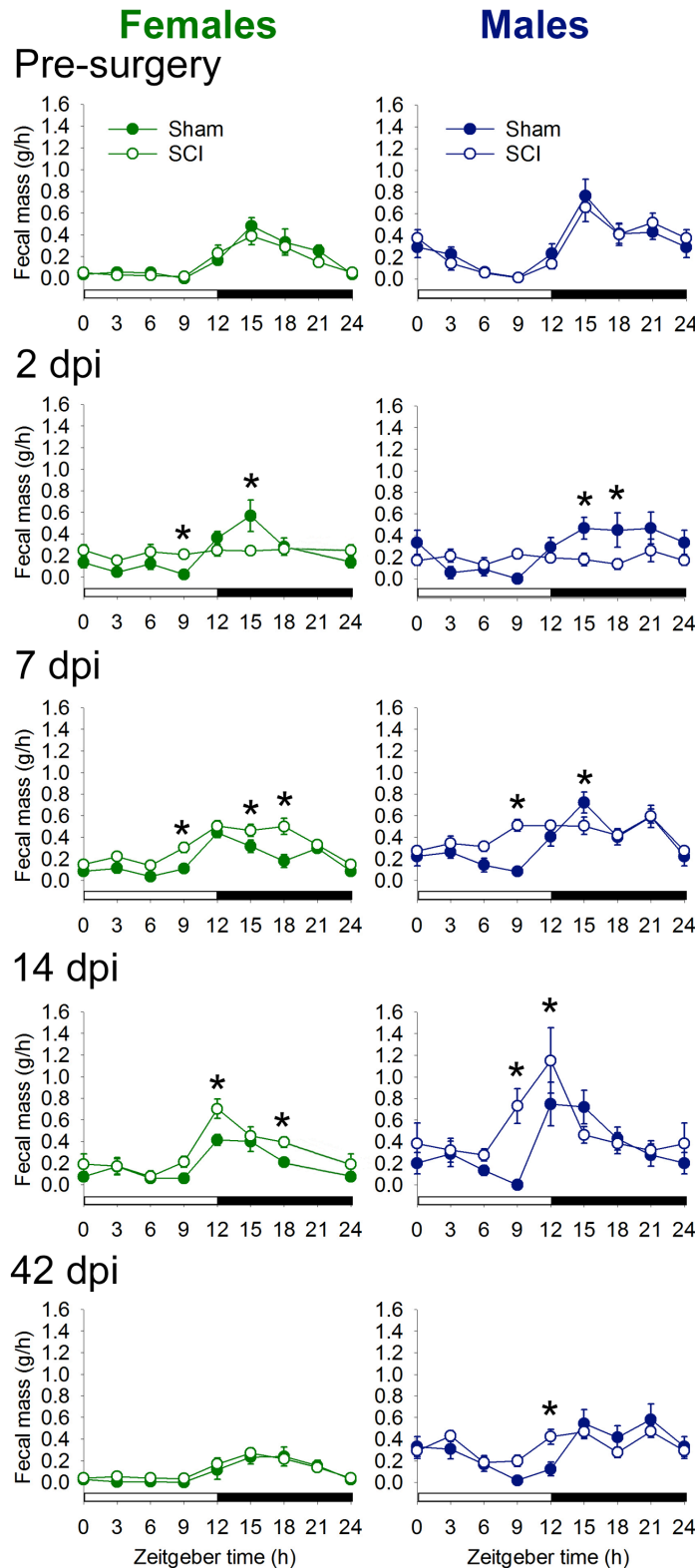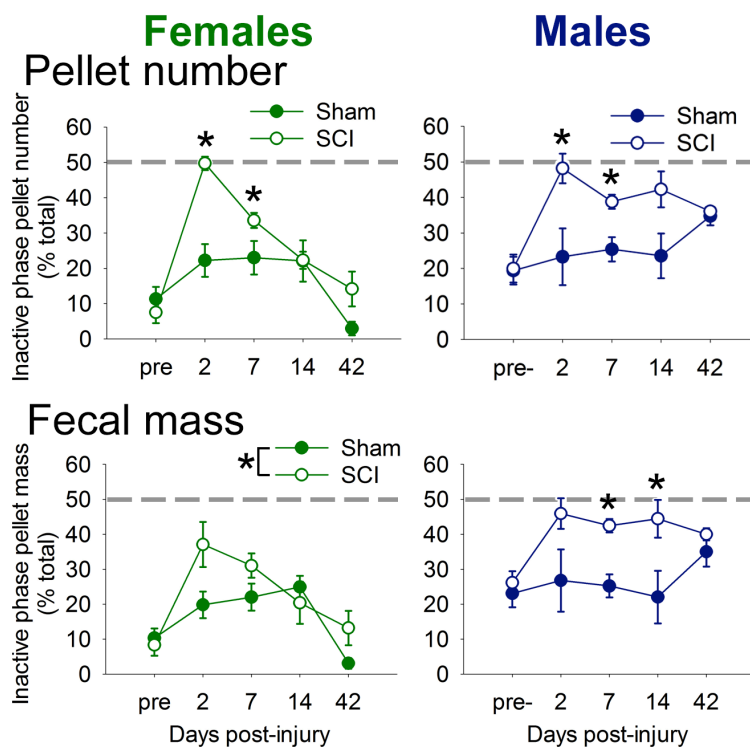
These SCI-elicited circadian differences are highlighted by examining the pellet number and mass output during the entire inactive phase (the time when healthy rats are typically excreting fewer pellets) (Fig. 2.6). Prior to injury, SCI rats produced few pellets in their inactive phase (females: $8 \pm 3\%$ pellets in inactive phase; males: $20 \pm 4\%$ pellets in inactive phase). At acute times post-SCI (2 and 7 dpi), female and male rats produce 35-50% of their pellets during their inactive phase (grey dashed line = 50% produced in inactive phase) (main effect of treatment: female: $F(1,27)=12.58$, $p < 0.001$; male: $F(1,27)=12.27$, $p = 0.001$) (female, sham v. SCI: 2 dpi, $p = 0.01$; 7 dpi, $p < 0.05$) (male, sham v. SCI: 2 dpi, $p < 0.05$; 7 dpi, $p < 0.01$). Thus, the daily rhythmic production of fecal matter in rats is completely abolished soon after SCI, and appears to recover by the chronic 42 dpi timepoint.

Fig. 2.4. Circadian timing of fecal mass output is altered after SCI in female and male rats. Fecal mass was recorded every 3 h for 24 h prior to surgery and at several post-surgery timepoints. SCI in females and males disrupted rhythmic fecal mass output at 2, 7, and 14 dpi (unusually high output during the inactive [light] phase, ZT 0-12; reduced output during the active phase, ZT12-24). In males, disrupted rhythms persisted at 42 dpi.

SCI alters daily rhythms in body temperature and activity
Next, we sought to determine whether SCI has more general effects on circadian rhythms. Female and male rats were implanted with small transmitters (MiniMitters) that remotely transmit body temperature and activity data to a computer. After recovery and one week of pre-surgery testing, rats were subjected to sham surgery (n=6 females and n=6 males) or T9 contusion SCI (150 kDyn, 1 s dwell; n=6 females and n=6 males). Body temperature and activity data were recorded in 30 min bins, and data were recorded from acute to chronic times post-surgery. Consistent with data showing that SCI disrupts circadian rhythms in fecal output, our preliminary analyses suggest that SCI disrupts daily rhythms in body temperature and activity (Fig. 2.6). These findings could have important implications for humans with SCI: they suggest that SCI may disrupt overall circadian rhythms, which could be detrimental for recovery. Further, our data could suggest that sleep is dysregulated at acute times after SCI. Discovering treatments that strengthen circadian rhythms could help expedite recovery after injury.

These analyses are ongoing and will be completed and included in our publication.

Fig. 2.5. SCI in female and male rats robustly disrupts fecal output rhythms, as measured by fecal pellet number (top panels) and fecal mass (bottom panels). At 2 dpi, SCI rats produce ~50% of fecal pellets during their inactive phase. The percent of pellets produced in the inactive phase remains elevated at 7 dpi. Fecal pellet mass production during the inactive phase is also increased in SCI rats; males show specific increases in inactive phase percent mass at 7 and 14 dpi.

Spinal cord injury may alter inflammatory state in the bowel
SCI disrupts bowel function, including expedited whole-gut transit time, increased fecal output, and disrupted circadian fecal output. It is possible that SCI disrupts bowel function by altering cell reactions and activity in the bowel. To examine this possibility, proximal and distal colons were collected from rats at 56 d post-SCI (rats also used in activity experiment above to limit rat use). Colons are currently undergoing PCR and immunohistochemical analyses to establish whether inflammation is altered in the bowel. Inflammatory mRNA markers (e.g., IL-1b, TNFa, IL-6, IL-10, Arginase-1) and protein localization (e.g., Iba1 to visualize macrophage density) will be examined. These analyses are nearly complete.
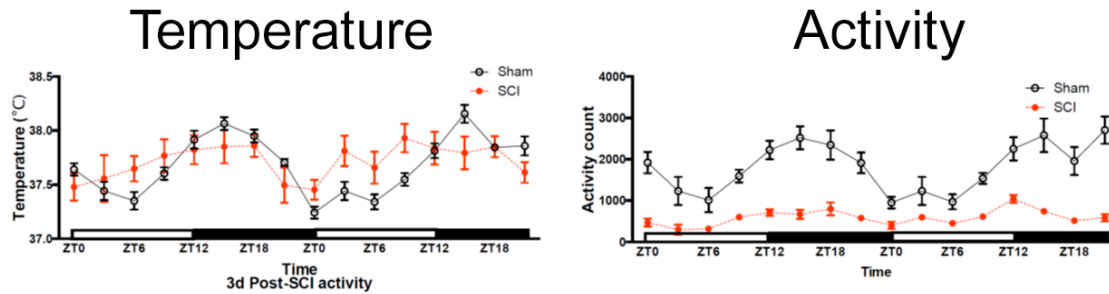
Fig. 2.6. Preliminary analyses indicate that SCI may cause more widespread circadian rhythm disruptions. Rats were implanted with transmitters that constantly recorded body temperature and activity. It appears that there are early SCI-induced alterations in daily rhythms of body temperature and activity (male rats at 2-3 dpi shown). Data for sham and SCI, male and female rats have been collected and are being analyzed.

KEY RESEARCH ACCOMPLISHMENTS
- Published an article on SNAP SCI, and the effects of morphine/(+)-naltrexone on neuropathic pain in *Brain, Behavior, and Immunity*.
- Presented new research on contusion SCI-induced pain and bowel dysfunction at two recent symposia.
- Presented poster highlighting contusion SCI-induced bowel dysfunction at an international conference: Society for Neuroscience in San Diego.
- Preparing manuscripts: one on contusion SCI-elicited neuropathic pain in male and female rats; another on post-SCI bowel dysfunction and circadian disruption.

CONCLUSIONS
Together, we have established CNS injury models that create neuropathic pain, and have revealed acute post-SCI fecal dynamics for the first time. In our recently-published *Brain, Behavior, and Immunity* paper (Ellis et al., 2016), we showed that T13 dorsal root avulsion ("SNAP surgery") elicited robust mechanical allodynia, that morphine exacerbated later SNAP-induced chronic pain, and that (+)-naltrexone alleviated this morphine-exacerbated pain. In contusion SCI studies performed this past year (soon to be submitted for publication), we examined neuropathic pain after several models of SCI: midline T9 contusion (no dwell), midline T9 contusion (1 s dwell), and unilateral T9 contusion. After all SCI types, mechanical allodynia developed; thermal hyperalgesia developed after the midline (but not unilateral) contusions. We predicted that morphine would worsen later chronic post-contusion pain (as with SNAP surgery); however, this was not observed. Finally, we examined fecal output after SCI and revealed that SCI accelerated whole-gut transit times, increased fecal output, and disrupted typical circadian rhythms of fecal output. Morphine treatment extended post-SCI whole-gut transit time. These data on post-SCI bowel dysfunction will be submitted for publication within the next 1-2 months.

Spinal cord injury neuropathic pain in rats
Through the support of the Department of Defense, we developed the contusion model in our lab and studied potential models of neuropathic pain. First, a typical midline contusion (no dwell

time; moderate- or moderate-severe injury severity) at T9 was tested. Modest neuropathic pain was observed with both injury severities; however, the moderate-severe injury caused more robust locomotor deficits that often precluded defining sensory thresholds. We sought to define another SCI model that may elicit more robust neuropathic pain symptoms (but still enabled sensory testing), so a midline moderate T9 contusion (with 1 s dwell time) was performed in female and male rats. This was completed in combination with acute morphine treatment (2x per d for 7 d), to determine whether morphine exacerbates SCI-induced pain as has been found in the SNAP CNS injury model. The midline T9 contusion with dwell successfully elicited neuropathic pain; however, morphine did not exacerbate later chronic pain. To establish whether morphine could exacerbate chronic pain in another injury type, we tested another potential SCI neuropathic pain model: T9 unilateral contusion SCI. This injury model causes robust pain when administered at C5 (Detloff et al., 2013;Putatunda et al., 2014); here, we adapted the unilateral injury to T9 to maintain continuity with our other data and to enable below-level sensory testing in the hindpaw. Unilateral contusion SCI caused mechanical allodynia in both ipsilateral and contralateral hindpaws. Acute 7 d course of morphine or (+)-naltrexone both had modest pain-provoking effects, particularly at 7 dpi.

Our data show that several rat SCI models can cause below-level neuropathic pain. This is consistent with previous studies (e.g., (Detloff et al., 2013;Gwak et al., 2012;Detloff et al., 2008).

Surprisingly, acute course of morphine treatment did not exacerbate later neuropathic pain. This has been shown in several models, including peripheral inflammatory nerve injury (chronic constriction injury) and spinal root avulsion injury. There are several possibilities that could explain why morphine-exacerbated pain was not observed. First, the mechanisms of pain at the lumbar level following contusion SCI versus other models may be different. Second, the transmission of CNS pain signals (excitatory, modulatory, inhibitory) is likely more severely influenced by SCI than by other models, which could alter responses to morphine. Third, the location of the injury relative to the area of sensory testing could influence results: SNAP surgery was completed at T13, which is closer to the hindpaw sensorimotor centers at spinal L4-L5. Thus, it is possible that receptors that potentially mediate morphine pain (e.g., TLR4) (Ellis et al., 2016;Eidson and Murphy, 2013) are not induced in lumbar spinal cord by T9 injury. It was clear that the morphine used was functional, as rats displayed typical post-morphine injection analgesia and tolerance.

Spinal cord injury causes bowel and circadian dysfunction in female and male rats
Bowel dysfunction is a major physical and psychological concern for individuals with SCI. Most studies on humans have examined bowel dysfunction at chronic times; however, a recent study showed that 92% of acutely-injured patients present with bowel dysfunction (Squair et al., 2015). This could affect post-SCI nutrient absorption, recovery, and sleep. Thus, our strategy for studying SCI-induced bowel dysfunction in rats could lead to a better understanding of post-SCI processes (presented at local and international meetings; soon to be submitted for publication).

Our data show that female and male SCI rats have expedited whole-gut transit time (time required for blue dye to pass from stomach into feces). This could correspond to incontinent-like symptoms. To our knowledge, this incontinent-like acute post-SCI phenotype has not been reported; it would be interesting to compare this to humans with SCI. This phenomenon could

also be due to the injury level and severity. In humans, injury level and severity influences the type and severity of bowel dysfunction. Individuals with more severe SCI and/or with injuries in the thoracic/cervical spinal cord are more likely to experience neurogenic bowel dysfunction (including constipation or fecal incontinence) (Liu et al., 2010;Valles et al., 2006). Patients with injuries at any level were susceptible to constipation; patients with injury below T7, when lacking sympathetic function, were more susceptible to fecal incontinence (Valles et al., 2006). Our injury was performed at T9, so it may be expected that incontinent-like symptoms were observed. Future studies could determine whether similar level-dependent patterns exist after SCI in rats (e.g., more constipation after SCI above T7, versus more incontinence after more caudal SCIs).

Circadian disruption was also observed after SCI. In fecal output studies with female and male rats, this circadian disruption was most robust at 2 dpi: rats produced ~50% of their pellets during their inactive phase. Circadian rhythms in fecal output were approximately normalized by 42 dpi (which is a chronic timepoint). Thus, it appears that moderate SCI causes acute circadian disruption that eventually resolves. To determine whether this generalizes to other circadian events, circadian rhythms in activity and body temperature were recorded. Our preliminary results suggest that our moderate T9 contusion SCI causes transient circadian disruption. This could have important implications for post-SCI recovery and rehabilitation. Further, it may signal that sleep quality is diminished after SCI (e.g., (Biering-Sorensen and Biering-Sorensen, 2001). Future studies could identify how SCIs at different levels/severities affect circadian rhythms, and whether SCI affects sleep quality and duration at acute and chronic post-SCI times. Ultimately, these studies could lead to new effective treatments.

Conclusions
In summary, this grant has led to several important findings. First, our research showed that CNS injury (SNAP surgery) results in neuropathic pain that is exacerbated by acute morphine treatment. (+)-naltrexone can improve this morphine-worsened neuropathic pain. These findings were recently published in *Brain, Behavior, and Immunity*. Second, we studied contusion SCI models of neuropathic pain in rats – this is the gold standard rodent model of human SCIs. Several contusion SCI models were used; they showed that contusion SCI can cause below-level mechanical allodynia and thermal hyperalgesia. Morphine caused only limited chronic neuropathic pain in these models, and (+)-naltrexone did not improve SCI pain. Finally, we examined SCI-induced bowel dysfunction in female and male rats. SCI expedited whole-gut transit time and disrupted circadian rhythms. Morphine lengthened post-SCI whole-gut transit time. SCI disrupted bowel and circadian function, suggesting that improving post-SCI dynamics could help improve circadian rhythms, activity, and possibly sleep. The contusion SCI neuropathic pain and bowel dysfunction studies will be published soon. Future studies could use these models to reveal new treatments that improve pain symptoms, circadian function, and overall recovery in rodent models of SCI.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS
a.

Published: Ellis A, Grace PM, Wieseler J, Favret J, Springer K, Skarda B, Ayala M, Hutchinson MR, Falci S, Rice KC, Maier SF, Watkins LR. Morphine amplifies mechanical allodynia via TLR4 in a rat model of spinal cord injury. *Brain, Behavior, and Immunity.* 2016; 58: 348-356.

Manuscript in preparation: Gaudet AD, Ayala MT, Fonken LK, Bateman EM, Schleicher W, Smith EJ, Maier SF, Watkins LR. Spinal cord injury in rats disrupts circadian rhythms and bowel function.

Manuscript in preparation: Gaudet AD, Ayala MT, Fonken LK, Bateman EM, Schleicher W, Smith EJ, Maier SF, Watkins LR. Exploring spinal cord injury-elicited neuropathic pain in female and male rats.

b.
Poster at International Meeting: Gaudet AD, Ayala MT, Fonken LK, Maier SF, Watkins LR (2016) Spinal cord injury in rats disrupts bowel function and daily activity rhythms. (Society for Neuroscience Annual Meeting, San Diego, USA)

Presentation: Gaudet AD. Using the immune system to resolve paralysis and pain after spinal cord injury. Postdoctoral Research Symposium. Boulder, CO, 2016.
Outstanding Postdoc Award Finalist.

Presentation: Gaudet AD. Effects of spinal cord injury on rat circadian function. Annual Colorado Sleep and Circadian Research Symposium. Boulder, CO, 2016.

INVENTIONS, PATENTS AND LICENSES
Nothing to report

REPORTABLE OUTCOMES
We have identified an effective strategy for collecting and analyzing fecal output from rats over the course of 24 hours. This could be used to identify effective treatments that improve post-SCI fecal dynamics, nutrient absorption, and circadian rhythms.

OTHER ACHIEVEMENTS
Nothing to report

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APPENDICES

1. Please see attached publication derived from Department of Defense funding:
Ellis A, Grace PM, Wieseler J, Favret J, Springer K, Skarda B, Ayala M, Hutchinson MR, Falci S, Rice KC, Maier SF, Watkins LR (2016) Morphine amplifies mechanical allodynia via TLR4 in a rat model of spinal cord injury. Brain Behav Immun 58:348-356.

2. See attached poster supported by the Department of Defense and presented at the renowned Society for Neuroscience conference in San Diego, CA; 2016:
Gaudet AD, Ayala MT, Fonken LK, Maier SF, Watkins LR (2016) Spinal cord injury in rats disrupts bowel function and daily activity rhythms. (Society for Neuroscience Annual Meeting, San Diego, USA)

3. Here is the abstract for the poster presented at Society for Neuroscience:

Title: Spinal cord injury in rats disrupts bowel function and daily activity rhythms

Theme and topic: C.09.f. Spinal cord: Animal models and human studies

Keywords: Spinal cord injury, circadian rhythm, bowel dysfunction, autonomic nervous system, autonomic dysreflexia

Authors: Andrew D. Gaudet, Monica Ayala, Laura K. Fonken, Steven F. Maier, Linda R. Watkins

Abstract: In addition to conspicuous effects on sensorimotor function, spinal cord injury (SCI) disturbs more subtle systems of the body. Many individuals with chronic SCI are afflicted with bowel dysfunction (incontinence and/or constipation). Further, SCI could perturb circadian rhythms causing homeostatic disruption that hinders recovery. Improving post-SCI bowel regularity and normalizing circadian rhythms could enhance recovery and quality-of-life. Here, we hypothesize that SCI in rats causes bowel dysfunction and disrupts circadian rhythms. Female and male rats were subjected to moderate-to-severe T9 contusion SCI (or sham surgery). Fecal production was then assessed at acute-to-chronic post-SCI times. Our novel data show that female and male rats have disrupted fecal production at acute times after SCI. Rats with acute SCI had reduced whole-gut transit time, suggesting potentially deficient nutrient absorption. SCI rats also produced more fecal pellets than shams during the inactive phase, implying that they may have disrupted circadian rhythms. Ongoing experiments will establish whether SCI alters circadian activity in rats. These studies reveal a novel strategy for assessing post-SCI fecal production and circadian rhythms in rats, and suggest that SCI disrupts bowel and circadian function.

A Novel Approach for Effectively Treating SCI Pain, Improving Opioid Efficacy, and Preventing Opioid-Induced Constipation: Key Role of Toll-Like Receptor 4 (TLR4)

W81XWH-13-1-0277/SC120066

PI: Linda Watkins   **Org:** University of Colorado Boulder   **Award Amount:** $570,099.00
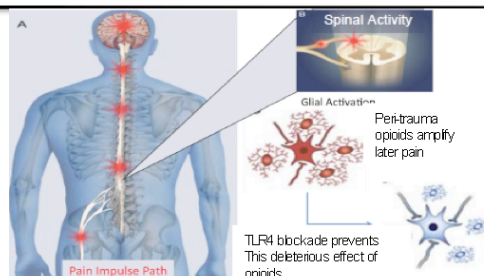
Study/Product Aim(s)

• **Aim I: Does co-administration of the TLR4 antagonist (+)-naltrexone with morphine prevent detrimental effects of morphine when this opioid is administered _shortly after_ dorsal root avulsion, during the acute stages of injury?**

• **Aim II: Does co-administration of the TLR4 antagonist (+)-naltrexone with morphine prevent detrimental effects of morphine when morphine is administered _weeks after (6 weeks)_ dorsal root avulsion, after _development of below-level neuropathic pain_?**

• **Aim III: Do robust phenomena from above generalize to the classic, widely employed SCI contusion model?**

Approach

Here we will attempt to prevent morphine amplification of spinal cord injury pain in two different models of SCI (dorsal root avulsion and contusion) by co-administering a selective TLR4 antagonist, (+)-naltrexone, with morphine at various timepoints post-surgery.

Spinal Activity
Glial Activation
Peri-trauma opioids amplify later pain
TLR4 blockade prevents This deleterious effect of opioids
Pain Impulse Path

Accomplishments:
• Published an article on SNAP SCI, and the effects of morphine/(+)-naltrexone on neuropathic pain in Brain, Behavior, and Immunity.
• Presented research on SCI-induced pain and bowel dysfunction at 2 recent symposia.
• Presented poster at an international conference: Society for Neuroscience in San Diego.
• Preparing two manuscripts: one on SCI pain; another on post-SCI bowel dysfunction.

Timeline and Cost

Activities	CY	13	14	15	16
Impactor Training/Purchase		▉	▉		
Aim I			▉		
Aim II				▉	
Aim III				▉	▉
Estimated Budget ($K)		$10	$185	$185	$185

Updated: 12.12.2016

Goals/Milestones
CY13 Goal – Purchase MACSIS contusion impactor
X Purchase and set-up impactor (Infinite Horizon)
CY14 Goals – prevent deleterious effects of morphine in acute avulsion SCI
X Prevent SCI pain
X Prevent motor deficits, enhance morphine analgesia
☐ Prevent constipation
CY15 Goal – Prevent deleterious effects of morphine in chronic avulsion SCI
☐ Prevent SCI pain
☐ Prevent motor deficits, enhance morphine analgesia
☐ Prevent constipation
CY16 Goal – Determine if robust phenomena from above generalize to contusion SCI
O Prevent SCI pain _(preparing to publish)_
O Prevent motor deficits, enhance morphine analgesia
O Prevent constipation _(preparing to publish)_

Comments/Challenges/Issues/Concerns
• X = complete; O = ongoing current studies
• Developed contusion SCI models that cause neuropathic pain
• Male and female rats display SCI pain that was not modified by morphine
• Discovered that SCI causes acute bowel dysfunction and circadian disruption

Budget Expenditure to Date
Projected Expenditure: $570,099
Actual Expenditure: $547,164 (No-cost extension granted)