

ANALGESIA OR ADDICTION: IMPLICATIONS FOR MORPHINE USE AFTER
SPINAL CORD INJURY

A Thesis

by

SARAH ANN WOLLER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2010

Major Subject: Psychology

Analgesia or Addiction: Implications for Morphine Use After Spinal Cord Injury

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Approved by:

Chair of Committee,	Michelle Hook
Committee Members,	James Grau
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ABSTRACT

Analgesia or Addiction: Implications for Morphine Use After Spinal Cord Injury. (May 2010)

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Chair of Advisory Committee: Dr. Michelle A. Hook

Up to 65% of individuals with a spinal cord injury (SCI) experience neuropathic pain, and cite this as one of the most significant consequences of injury. Opiate analgesics are one of the most effective, but also most concerning, treatments for neuropathic pain. In fact, the use of morphine after SCI can potentiate the development of paradoxical pain symptoms, and continuous administration can lead to dependence, tolerance, and addiction. Empirical evidence suggests that the addictive potential of morphine decreases when used to treat neuropathic pain, but this has not been studied in an SCI model. These studies, therefore, aimed to investigate the addictive potential of morphine in a rodent model of spinal contusion injury.

These experiments used a conditioned place preference (CPP) paradigm to examine whether subjects with SCI would develop a preference in the acute phase of injury, and whether a place preference would be expressed after the development of neuropathic pain symptoms in the chronic phase of injury. Results suggest that the time of treatment did affect the development of a preference for the morphine-paired context; subjects displayed a CPP in the acute, but not the chronic phase of SCI. In addition, the

findings indicate that spinal neurons are sufficient, but not necessary, for producing a morphine-induced place preference.

Overall, the results suggest that morphine could be used for the clinical treatment of neuropathic pain without concerns of addiction. Although SCI alone did not reduce the “addictive” potential of morphine in the acute phase of injury, the lack of preference in the chronic phase suggests that addiction may be reduced by molecular changes that accompany the development of neuropathic pain. Moreover, we hypothesize that the analgesic effects of morphine acting on spinal and peripheral mu-opioid receptors (MOR’s) underlies the development of CPP in the acute phase of injury. This hypothesis is supported by the CPP established with intrathecal morphine administration. Nonetheless, the current studies cannot discount the role of supraspinally-mediated reward in the development of place preference after injury. Further work is needed to distinguish between the addictive and analgesic properties of morphine.

DEDICATION

This thesis is dedicated to my mother, Sharon A. Bertling. My work would not have been possible without her constant support, guidance, and reminders to “follow your dreams”.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Michelle A. Hook for her supervision, support, and patience. In addition, I wish to thank my committee members, Dr. James W. Grau and Dr. Rajesh Miranda for their guidance throughout the course of this research.

To my fellow members of the Grau Lab: Thank you. It has been my pleasure sharing lab space with you.

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Gina, while you have left Texas A&M to pursue your own graduate career, you were always a cheerful presence in the lab, and made me smile on bad days. My gratitude for your help on these projects cannot be expressed adequately in words.

Kyle, I must thank you for always taking time to answer my questions, and for helping me manage life as a graduate student. I appreciate all of your assistance.

Finally, thanks to my family for their encouragement, love, and support. Having a Thanksgiving in Texas each year has become a great family tradition, and has made each fall semester better.

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INTRODUCTION

Neuropathic pain is one of the most significant consequences of spinal cord injury (SCI), and is one of the primary symptoms patients would like to have effectively treated (Anderson 2004; Backonja & Stacey 2004). Approximately 65% of individuals with SCI experience pain, and the majority describe the pain as severe or excruciating (Perry et al. 2008; Budh & Lundeberg 2005; Siddall et al. 2003). Unfortunately, typical pain relievers are often ineffective in treating this pain after SCI, and the pain tends to get worse with time rather than better (Katz et al. 2008; Budh & Lundeberg 2005; Zaho et al. 2004). Moreover, it has been demonstrated in both animal models and human studies that administration of analgesics, such as morphine, can potentiate the development of neuropathic pain, allodynia, and hyperalgesia (Liang et al. 2008; Chang et al. 2007; Hook et al. 2007; Parisod et al. 2003; Yu et al. 1997a&b). Considering the number of people that neuropathic pain affects, it is important that it can be effectively treated.

Opiates are commonly used for the treatment of neuropathic pain after SCI (Sindrup & Jensen 1999; Liang et al. 2008; Przewlocki et al. 2005; Clark 2002; Warms et al. 2002; Widerstrom-Noga & Turk 2003; O'Conner & Dworkin 2009), and are considered to be among the most effective analgesics (Warms et al. 2002). From a clinical perspective, however, the prescription of opiates is concerning because continuous administration can lead to dependence, tolerance, and addiction (Trescot et al. 2008; Ballantyne & Mao 2003; O'Conner & Dworkin 2009). It is estimated that 18-

This thesis follows the style and format of *Behavioral Neuroscience*.

45% of individuals using opioids for the management of chronic pain, or pain that lasts beyond the usual course of disease or healing, abuse the drug (Contet et al. 2008; Trescot et al. 2008; Compton & Volkow 2005; Heinemann et al. 1992; Morasco & Dobscha 2008). Contradictions between reports on the incidence of addiction, resulting in the large reported range, stem from different definitions of abuse, methods of reporting, populations being surveyed, and a general lack of empirical research examining the efficacy of long-term opioid use and addictive potential (Morasco & Dobscha 2008; Dersh et al. 2008; Bell & Salmon 2009; Radnitz & Tirch 1995; Hojsted & Sjogren 2007). Clearly, the use of opioids for the management of pain must be further investigated.

While addiction is seen in individuals using opiates for the treatment of chronic pain, studies of human and animal subjects suggest that there is a low risk of abuse in neuropathic pain conditions (Martin et al. 2007; Ballantyne & Mao 2003; Clark 2002; Vetulani 2001). Thus, the addictive potential for opiates following chronic pain, or pain that lasts beyond the usual course of a disease, may differ from the addictive potential in conditions of neuropathic pain, or pain resulting from a nerve injury. Supporting the claim that neuropathic pain results in decreased addictive potential for opiates, Bardo et al. (1986) showed that morphine is capable of inducing a place preference after a single pairing in intact rats, but morphine will not produce a conditioned place preference (CPP) in rats experiencing neuropathic pain resulting from nerve ligation (Ozaki et al. 2002). Moreover, Lyness et al. (1998) found that arthritic rats self-administered significantly less morphine than their pain-free counterparts. These studies suggest that

morphine will not be addictive after SCI. However, a decreased addictive potential after SCI cannot be assumed. Studies have clearly demonstrated that opiates have differential effects that depend on the model, the pain assessment tool, and the route of analgesic administration (Yu et al. 1997a&b). Furthermore, if prior behavior is predictive of addictive potential, one might expect an increased incidence of addiction after SCI. Heinemann et al. (1988) found that up to 62% of SCI patients had misused drugs or alcohol at the time of their injury. Finally, the prevalence of opiate abuse in patients with chronic back pain underscores the need to further examine addiction with the treatment of neuropathic pain after SCI.

To address this issue, the current studies investigate the addictive potential of morphine in a rodent contusion model of SCI. The contusion model closely resembles the clinical condition of SCI (Hulsebosch 2002), producing symptoms of chronic pain in approximately 80% of subjects (Mills et al. 2001). Rats show signs of thermal, mechanical, and girdle allodynia around two weeks following the contusion injury (Hulsebosch et al. 2000). Using this model we examined whether subjects with SCI would develop a CPP in the acute phase of injury (Experiment 1), and whether this would differ with the development of neuropathic pain symptoms in the chronic phase of injury (Experiment 2). Interestingly, we found that the time of treatment did affect the development of a preference for the morphine-paired context; subjects displayed a CPP in the acute but not the chronic phase of SCI. Experiments 3 and 4 examined the necessity and sufficiency of spinal neurons in the development of a CPP. The results indicate that spinal neurons are capable of producing a morphine-induced place

preference, but this preference was not blocked by the intrathecal administration of naltrexone.

GENERAL METHODS

Subjects

Male, Sprague-Dawley rats obtained from Harlan (Houston, TX) were used as subjects. Animals were 90-110 days old (350-400 g), and were individually housed in Plexiglas bins [45.7 (length) x 23.5 (width) x 20.3 (height) cm] with food and water available *ad libitum*. Subject's bladders were expressed manually in the morning (8-9:30 a.m.) and evening (6-7:30 p.m.) until they regained bladder control, which was defined as three consecutive days with an empty bladder at the time of expression. Animals were maintained on a 12-hour light-dark cycle and all behavioral testing occurred during the light portion of the cycle.

All of the experiments were reviewed and approved by the institutional care committee at Texas A&M and all NIH guidelines for the care and use of animal subjects were followed.

Surgery

For the contusion injury, subjects were anesthetized with inhaled isoflurane (5% to induce anesthesia and 2-3% for maintenance), and an area approximately 4.5 cm above and below the injury site was shaved and disinfected with iodine. A 7.0 cm incision was made over the spinal cord, and two incisions extending 3 cm rostral and caudal to T12-T13 were made on either side of the vertebral column. The dorsal spinous processes at T12-T13 were removed (laminectomy), exposing spinal tissue. The vertebral column was then fixed within the MASCIS device (Constantini and Young 1994; Gruner 1992) and a moderate contusion injury was produced by allowing the 10 g

impactor (outfitted with a 2.5 mm tip) to drop 12.5 mm. The wound was closed with Michel clips. Sham subjects received a laminectomy only (no weight drop), and intact subjects received anesthesia only.

In Experiments 3 and 4, an intrathecal catheter was implanted immediately after the contusion injury. For this procedure, a 15-cm polyethylene (PE-10) cannula, fitted with a .23 mm (diameter) stainless steel wire (SWGX-090, Small Parts), was inserted into the subarachnoid space under the vertebrae and 2 cm caudal to the injury. The tubing was secured to the vertebrae rostral to the injury site with an adhesive (Cyanoacrylate) to prevent movement of the cannula. The wire was removed from the tubing, and the wound was closed with Michel Clips.

For the first 24 hours after surgery, rats were housed in a recovery room maintained at 26.6°C. All subjects were treated with 100,000 units/kg Pfizerpen (penicillin G potassium) immediately after surgery and again 2 days later. To help maintain hydration, subjects were also given 3.0 ml of saline (i.p. injection) following surgery. Michel clips were removed 14 days following surgery.

Assessment of Motor and Sensory Recovery

Locomotor Recovery. Locomotor behavior was assessed using the Basso, Beattie, and Bresnahan (BBB) scale (Basso et al. 1995) in an open enclosure (99 cm diameter, 23 cm deep) on the day following injury. Subjects were acclimated to the apparatus for 5-min per day for 3 days prior to surgery. Twenty-four hours after surgery each subject was placed in the open field and observed for 4-min to assess locomotor

behavior. All observers had high intra- and inter-observer reliability (all $r's > .89$) and were blind to the subject's experimental treatment.

Locomotor scores were transformed to help assure that the data were amendable to parametric analyses (Ferguson et al. 2004a). This transformation pools BBB scores 2-4, removing a discontinuity in the scale. The transformation also pools scores from a region of the scale (14-21) that is very seldom used for a moderate contusion injury. By pooling these scores, we obtain an ordered scale that is relatively continuous with units that have approximately equivalent interval spacing. Meeting these criteria allows us to apply metric operations (computation of mean performance across legs), improves the justification for parametric statistical analyses, and increases statistical power.

Mechanical Reactivity. Reactivity was assessed using von Frey stimuli formed from nylon monofilaments (Semmes-Weinstein Anesthesiometer; Stoelting Co., Chicago, IL) and applied to the plantar surface of the hindpaws. Subjects were placed into Plexiglas tubes [7.0 cm (internal diameter) x 20 cm (length)] that had 6 cm (length) x 1.7 (width) cm notches removed from the sides, to allow the hindlimbs to hang freely. After a 15-min acclimation period, the von Frey stimuli were applied sequentially at approximately 2 sec intervals until subjects withdrew the paw and vocalized. If no response was observed, testing was terminated at a force of 300 g. Each subject was tested twice on each foot in a counterbalanced ABBA order. Test sequences were spaced 2 min apart. Stimulus intensity was reported using the formula provided by Semmes-Weinstein: $\text{Intensity} = \log_{10}(10,000 * \text{g force})$.

Girdle Test. A grid map of the girdle zone for allodynic responding was made on the rats using an indelible marker (44 squares). A single von Frey filament with bending force of 204.14 mN (26 g force) was applied to each point on the grid, and vocalization responses were recorded on a grip map of that animal. For each subject, the total number of vocalizations were recorded (N_v) and normalized by the following formula: percent vocalizations= $(N_v \times 100)/$ total number applications (44).

Thermal Reactivity. Reactivity to a noxious thermal stimulus was assessed by applying radiant heat to the tail. A 375-W movie light was focused onto the subject's tail using a condenser lens positioned 8 cm below the light source. The subject's tail was positioned in a 0.5 cm deep groove cut into an aluminum block 4.7 cm below the condenser lens. The last 2.5 cm of the tail was taped to a wire hook and attached to an elastic band located 11 cm behind the aluminum block, exposing approximately 2 cm of the tail to the light source. The flexibility of the elastic band allowed for a tail flick response while maintaining the rat's tail under the heat source. The latency to vocalize was then assessed. After both movement and vocalization responses were detected, the heat was terminated. If a subject failed to respond, the test trial was automatically terminated after 8 s of heat exposure to avoid tissue damage. Subjects were placed in the apparatus for 15 min prior to testing and were assessed 3 times at 2 min intervals. The last two tests were averaged to derive a measure of reactivity.

Place Preference Procedure

Apparatus. Acclimation to the training/testing environments took place in grey plywood boxes [41cm (length) x 41cm (height) x 38 cm (width)] with smooth floors.

Morphine place preference conditioning occurred in one of two distinct environments. One box [41cm (length) x 41cm (height) x 38cm (width)] was black with a smooth Plexiglas floor scented with 3% vinegar. The other was a white box and the floor was covered with pine chips. The boxes were cleaned with a disinfectant (Novalsan) between subjects. Testing occurred in a box [91cm (length) x 41cm (height) x 38 cm (width)] that was comprised of both of the training contexts separated by a neutral grey strip. The conditioning boxes and test box were illuminated in a manner that eliminated the natural preference for the black or white portion of the box, and were maintained in the same position for the duration of the experiment.

Acclimation. The subjects were brought into the room and were placed into the grey acclimation boxes (described previously) for 45-min. This was done to familiarize rats with the handling, environment, and apparatus (see Figure 1A).

Baseline Preference. Animals were observed for 15-min in the testing box (with the black context and white context separated by a neutral grey strip) to assess baseline preference prior to training (see Figure 1C).

Training. As in previous designs (e.g. Ferguson et al. 2004b), training occurred in morning and afternoon (5-hrs later) sessions, allowing animals to experience both the drug and saline-paired context in the same day. Animals were given an injection of morphine or 0.90% saline, and were placed in a training context (black or white) for 45-min before being returned to their home cage for 5-hrs. In the afternoon session, subjects were injected with the other solution (rats that received saline with the first injection receive morphine in the second). Rats were then placed into the other context

for 45-min. In total, rats were given two training trials each day. One of these trials consisted of conditioning of the drug-paired context, and the other involved an injection of the vehicle followed by exposure to the vehicle-paired context (see Figure 1B). Both the order of presentation and which context served as the drug-paired environment were counterbalanced across individuals. Baseline preferences for the black/white context were balanced across groups.

Testing. On the day following the last training session, rats were placed in the testing chamber and observed for 15-min to assess time spent in the drug-paired context, neutral area, and saline-paired context (see Figure 1C). Testing occurred in the middle of the day (12:00-14:00 hrs).



Figure 1: A. During Acclimation, animals were placed into a grey box for 45-min. B. In Training, animals were given an injection of morphine or saline paired with one of two contexts: a black chamber with a smooth floor and scented with vinegar, or a white chamber with pine chips covering the floor. Animals experienced both contexts for 45-min on each of two days. C. Following two days of training, animals were placed in a large box with both contexts separated by a neutral grey strip. Animals were observed for 15-min to assess preference. This box was also used to assess baseline preference before any training occurred.

Data Analyses

All data were analyzed using an analysis of variance (ANOVA), with an *a priori* alpha value of .05. Place preference was analyzed by comparing the ratio of the time

spent in the morphine-paired side of the testing chamber to the time spent in the vehicle-paired context [morphine paired/(saline paired+1)].

EXPERIMENT 1

Opioid analgesics are one of the only effective pharmacological treatments available for neuropathic pain, but no research has been conducted to examine the addictive potential of these drugs after SCI. The aim of this experiment, therefore, was to examine whether a place preference for morphine can be established following a moderate contusion injury.

Procedure

Experiment 1 used 48 subjects (n=8) randomly assigned to one of two morphine sulfate (Sigma–Aldrich, St. Louis) dose conditions (1.25 or 2.5 mg/kg), and one of three surgery conditions (contusion, sham, or intact). Conditioned place preference training and testing occurred over 7-days (see Figure 2).

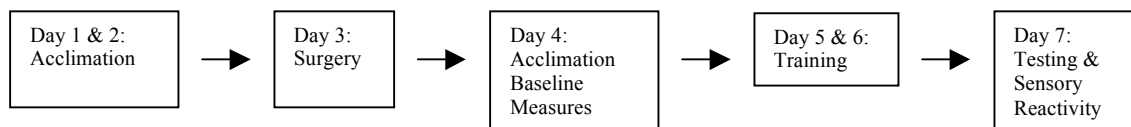


Figure 2. The timeline used for conditioning of the animals is depicted. Animals were acclimated for two days prior to surgery and one additional day following surgery when baseline measures of preference and sensory reactivity were also recorded. Training began after surgery, and testing started 4 days post injury. Following all CPP training and testing, sensory reactivity was reassessed.

Results

Baseline Measures of Motor and Sensory Reactivity. As expected, locomotor scores assessed on the day following surgery were significantly lower in contused subjects compared with sham and intact controls ($F_{(2,42)}=405$, $p<.05$). As seen in Figure

3, contused subjects had converted BBB scores indicative of a moderate contusion injury; 3.19 ± 0.06 in the 1.25 mg/kg group and 4.25 ± 0.50 in the 2.5 mg/kg morphine group. Sham subjects in both groups had BBB scores of 12 ± 0.00 , indicating that they displayed consistent plantar placement of the hindpaws and a coordinated gait.

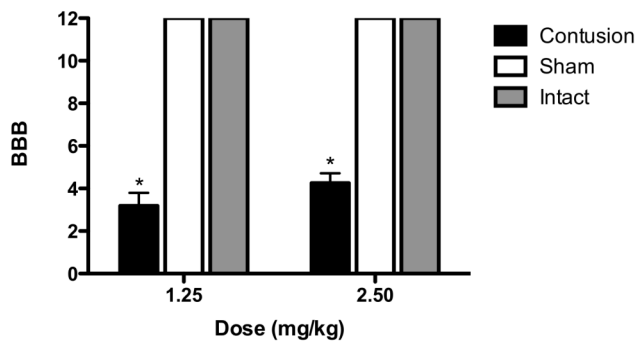


Figure 3. Animals in the contusion groups showed converted BBB scores indicative of a moderate contusion injury. Sham controls did not differ from Intact controls. * $p < .05$

Subjects were initially assigned to groups based on Day 1 BBB scores.

However, there were some differences between groups in baseline assessments of sensory reactivity. There was a significant main effect of Surgery on the von Frey tactile reactivity task ($F_{(2,42)}=5.71$, $p < .05$; see Figure 4A). Duncan new multiple-range *post hoc* analyses confirmed that contused subjects were significantly less reactive than sham subjects ($p < .05$). There was also a significant main effect of assigned Dose on tactile reactivity ($F_{(1,42)}=14.77$, $p < .05$), and an assigned Dose x Surgery interaction ($F_{(2,42)}=4.43$, $p < .05$). Subjects in the 2.5 mg/kg morphine group were initially less reactive than those in the 1.25 mg/kg group. For the supraspinal measure of vocalizations in response to

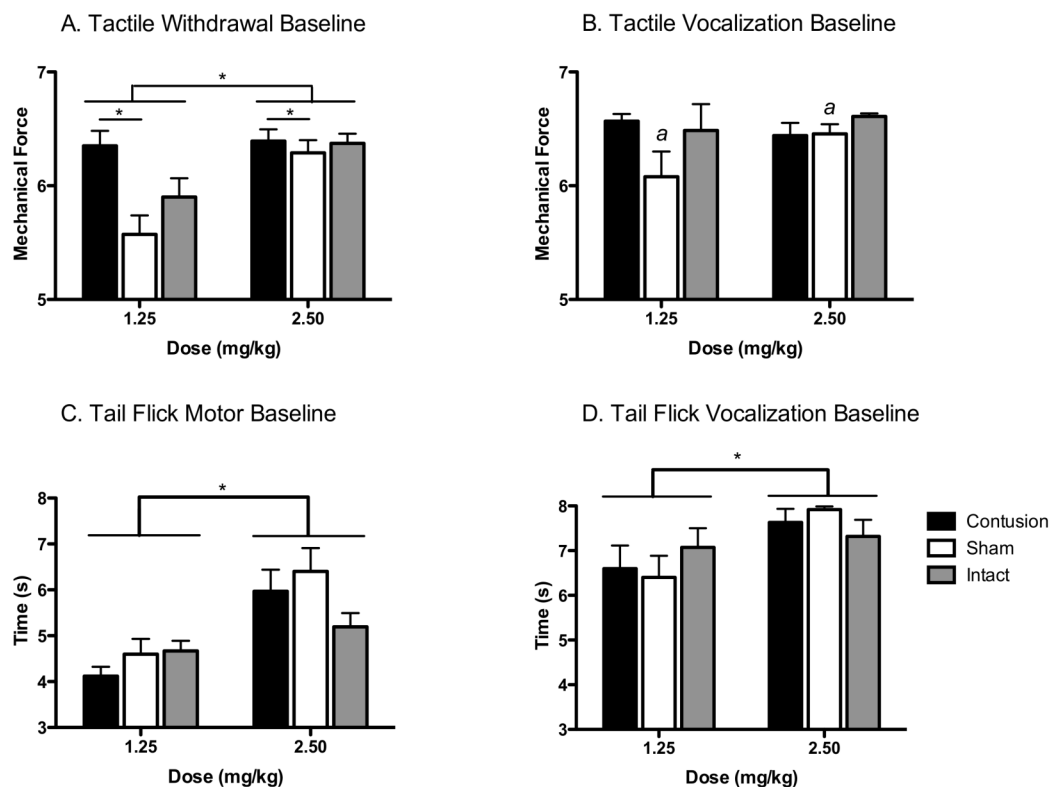


Figure 4. For both motor and vocal reactivity, contused subjects displayed higher reactivity thresholds than sham controls (A and B respectively). These data likely reflect the loss of neural function in the acute phase of injury. For motor and sensory reactivity to heat, there was also a significant main effect of assigned Dose. Subjects assigned to the 1.25mg/kg group displayed lower reactivity thresholds, with a faster tail flick (C) and vocalization (D) response; * $p < .05$, a indicates sham animals were significantly different from contusion and intact subjects.

tactile stimulation, there was a significant main effect of Surgery ($F_{(2,42)}=3.39$, $p < .05$; see Figure 4B). Sham subjects vocalized at lower von Frey thresholds than contused and intact subjects. To control for these differences in baseline reactivity, a change from baseline score (response after place preference training-response prior to place

preference training) was calculated for subsequent comparisons of reactivity thresholds across groups.

As found on the tests of tactile reactivity, there was a significant main effect of assigned Dose on both motor responses and vocalizations elicited by a radiant heat stimulus (motor: $F_{(1,42)}=23.16$, vocalization: $F_{(1,42)}=8.37$, $p's < .05$; see Figure 4 C & D). Subjects assigned to the 2.5mg/kg group had longer latencies to exhibit a tail flick and to vocalize in response to stimulation than subjects assigned to the 1.25mg/kg group. To control for these baseline differences, a change from baseline score was again used in subsequent analyses.

Assessment of Place-Preference. There was a main effect of Surgery condition on place preference ($F_{(2,42)}=5.06$, $p < .05$). As displayed in Figure 5, contused subjects showed a significantly stronger preference for the morphine-paired context, for both drug doses, compared to intact and sham controls. There was no effect of drug Dose, or a Dose X Surgery interaction, on the place preferences observed. Pearson's Product Moment Correlations were used to examine whether any of the baseline reactivity measures correlated with preference. Results indicate neither tactile withdrawal or vocalizations nor tail flick motor response or vocalizations correlated with the CPP (all $r's < .27$).

Assessment of Sensory Reactivity After Place Preference Training. Using the corrected (change from baseline) index, there were no significant differences between groups on the motor response to von Frey stimulation in the tactile reactivity task ($F_{(2,42)} < 1.0$, $p > .05$). There was, however, a significant main effect of surgery condition

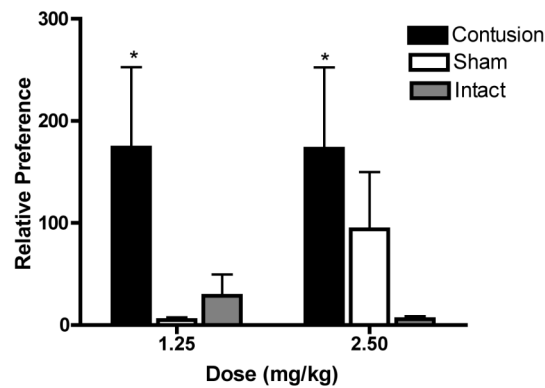


Figure 5. Assessment of the CPP revealed a significant effect of surgery condition. Contused subjects showed an increased preference for the morphine-paired context relative to sham and intact controls; * $p < .05$.

when vocalizations in response to tactile stimulation were examined ($F_{(2,42)}=5.10$, $p < .05$). The contused and sham subjects both differed from intact controls ($p < .05$). Intact controls vocalized at lower thresholds after place preference testing (Figure 6). There were no significant differences across groups on the tests of thermal reactivity.

To test whether morphine continued to produce analgesia after repeated administrations, all subjects were also given an i.p. injection of morphine (their previously assigned dose) and thermal reactivity was re-assessed. Analyses revealed no differences between groups, confirming that both doses of morphine produced significant analgesia, relative to vehicle, across groups (motor: $F < 1.0$, vocal: $F < 1.0$, both p 's $> .05$).

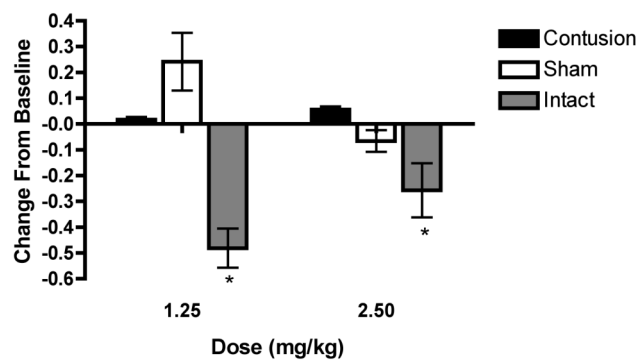


Figure 6. Following training and testing, we found a significant difference in vocalizations in response to a tactile stimulus. Intact controls vocalized at lower thresholds than sham and contused subjects * $p < .05$.

Discussion

Immediately following injury, contused subjects given 1.25 or 2.5 mg/kg i.p. morphine showed an increased preference for the morphine-paired context relative to sham and intact controls. This suggests that a contusion injury significantly increases the addictive potential of morphine in the acute phase of injury.

EXPERIMENT 2

Previous work has suggested that neuropathic pain decreases the addictive potential of morphine in a nerve injury model (Suzuki et al. 1996; Ozaki et al. 2002; Martin et al. 2007). Experiment 1 showed, however, that the addictive potential of morphine increased in the acute phase of injury. We hypothesize that, as symptoms of neuropathic pain do not develop until 14 days following a contusion injury, the addictive potential of morphine may not decrease until the more chronic phase of SCI. Experiment 2 examined whether there is a ‘window of vulnerability’ during which subjects are more likely to show a conditioned place preference for morphine following SCI.

Procedure

This experiment used 60 subjects (n=10) randomly assigned to one of six groups in a 2 (day 2 or 14) x 3 (intact, sham, or contusion) experimental design. Subjects were conditioned on two separate timelines. One group of subjects replicated the timeline from Experiment 1 using a 2.5 mg/kg dose. The second group experienced delayed training and testing, so that training began 14 days following surgery (Figure 7).

Results

Baseline Measures of Motor and Sensory Reactivity. Locomotor scores for contused subjects were, again, indicative of a moderate injury and were significantly different from sham controls ($F_{(1,36)}=632.33$, $p<.05$). Also, scores for the Day 1 and Day 14 contused groups did not differ. Mean BBB scores (\pm SEM) collected on the day

following surgery were 2.95 ± 0.56 for the Day 2 group and 3.45 ± 0.49 for the Day 14 group. All subjects in the sham group had converted BBB scores of 12 ± 0.00 .

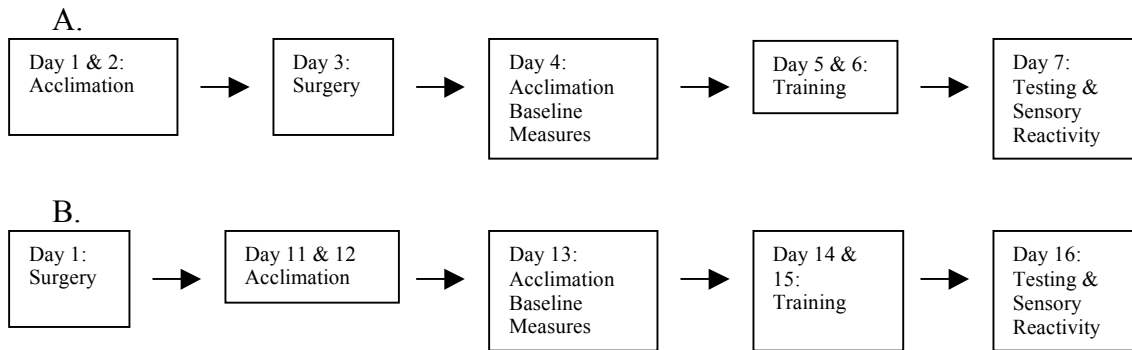


Figure 7. Conditioning of the animals occurred according to the timelines depicted above. A) This timeline replicates the conditioning schedule of Experiment 1 and was used for the Day 2 group. The Day 14 group was conditioned according to the timeline shown in B. Acclimation began 11 days following surgery, and baseline measures were taken on day 13 following surgery. Training began 14-days post-injury, and testing on day 16.

Similarly, there were no significant differences between the groups in baseline girdle reactivity or motor responses to tactile stimulation of the hindpaws. There was, however, a significant main effect of Surgery on vocal responses to tactile stimulation. As found in Experiment 1, sham subjects vocalized at lower thresholds than contused subjects ($F_{(2,54)}=4.58$, $p<.05$). Baseline tests of motor responses to the radiant heat stimulus also revealed a significant main effect of Surgery ($F_{(2,54)}=5.26$, $p<.05$), Day of testing ($F_{(1,54)}=10.89$, $p<.05$), and a Surgery x Day of testing interaction ($F_{(2,54)}=4.64$, $p<.05$). The intact groups had a longer latency to tail flick than the sham and contusion groups. In addition, animals in the 2-day group had significantly longer tail flick

latencies than the 14-day group. Vocalization thresholds did not differ across groups on the thermal reactivity test.

Assessment of Place-Preference. There was a significant main effect of the Day of testing on the amount of time spent in the drug-paired context ($F_{(1,54)}=5.10$, $p<.05$). As shown in Figure 8, contused subjects in the 2-day group showed an increased preference relative to intact controls, replicating the results of Experiment 1. However, none of the subjects in the 14-day group preferred the drug-paired context.

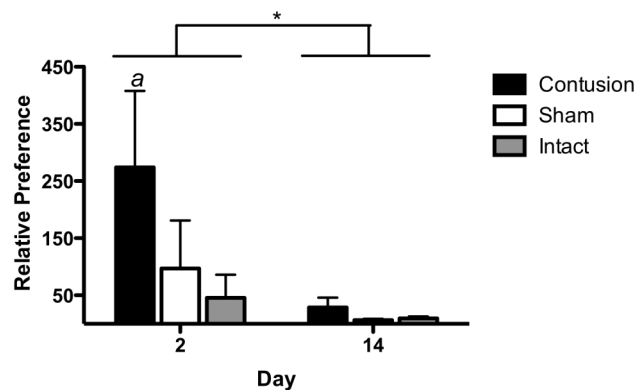


Figure 8. Results of the preference testing revealed a main effect of testing day. Contused subjects in the day 2 group replicated the results of Experiment 1 and showed a preference for the morphine-paired context (a). However, none of the subjects in the 14-day group preferred the drug-paired context.

Assessment of Sensory Reactivity After Place Preference Training. As predicted, contused subjects at 14 days displayed significantly higher levels of girdle reactivity compared to all other groups ($F_{(1,36)}=6.69$, $p<.05$), reflecting the development

of neuropathic pain (Figure 9A). Conversely, however, subjects in the 14-day group exhibited a longer latency to tail flick after place preference training ($F_{(1,54)}=4.23$, $p<.05$; Figure 9B). These seemingly disparate results may reflect differences in the type of pain experienced as well as differential modulation of reactivity at spinal and supraspinal loci. A tail flick to a radiant heat stimulus can be elicited after a spinal transection. The decreased reactivity to heat, therefore, may reflect an intraspinal modification resulting from the loss of motor neurons at the level of injury. By contrast, girdle reactivity may be modulated by spinal or supraspinal processes above the injury. Indeed, there were no group differences in latency to vocalize to the radiant heat stimulus. There were also no differences across groups when motor and vocal responses to von Frey stimulation of the hindpaws were assessed.

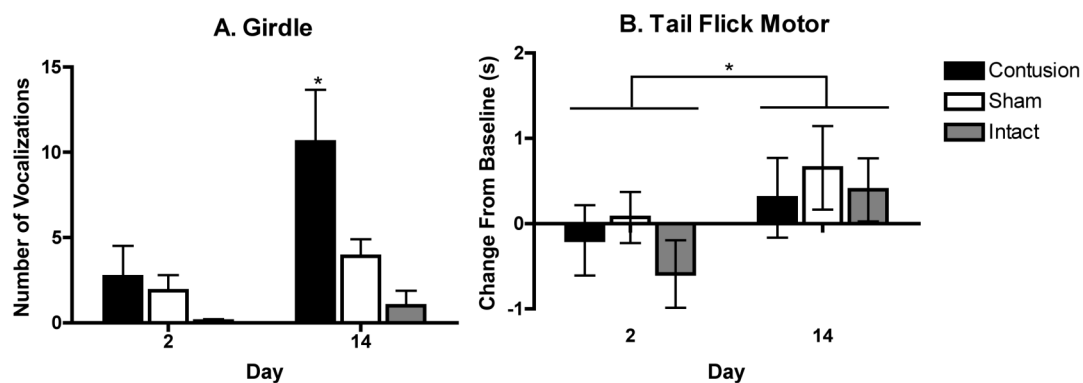


Figure 9. A.) On Day 14, contused subjects displayed signs of neuropathic pain, with significantly higher levels of girdle reactivity compared with all other groups. B.) Animals in the Day 14 groups showed longer tail flick latencies following morphine administration than did animals on Day 2. *p'<.05

At the end of place preference training and testing, the analgesic efficacy of morphine was reassessed. We found a significant main effect of Day ($F_{(1,54)}=11.01$, $p<.05$), and a significant Day x Surgery interaction ($F_{(2,54)}=6.90$, $p<.05$). Further analyses revealed animals in the Day 14 group had shorter tail flick latencies than did animals in the Day 2 group ($F_{(1,58)}=9.01$, $p<.05$), and *post hoc* analyses revealed this effect was driven primarily by Day 14 Intact animals ($p<.05$). It seems morphine administration, in the Day 14 Intact animals, did not produce robust antinociception, however, there were no differences between groups when examining vocalizations in response to the thermal stimulus ($p>.05$).

Discussion

Supporting previous studies, contused subjects that displayed symptoms of at-level neuropathic pain, did not develop a significant preference for the morphine-paired context. This suggests, that in the chronic phase of a moderate contusion injury, the addictive potential of morphine is attenuated.

EXPERIMENT 3

To determine whether non-spinal processes are sufficient to induce a CPP with morphine, we examined whether intrathecal naltrexone could block the antinociceptive and ‘addictive’ effects of systemic morphine.

Procedure

Animals were given a 7 μ g i.t. naltrexone injection 15-min prior to administration of an i.p. injection of 2.5mg/kg morphine. All place preference training and testing occurred as described previously (see Figure 2). To ensure that naltrexone was blocking antinociception at the spinal loci, sensory reactivity was assessed following each training, and testing, session. This experiment used 16 rats (n=8).

Results

Baseline Measures of Motor and Sensory Reactivity. There were no differences in mean converted BBB scores (\pm SEM) between groups on the day following surgery. Animals in the saline group had scores of 2.06 \pm 0.47 and animals in the naltrexone group had scores of 2.00 \pm 0.39. In addition, there were no baseline differences in tests of girdle reactivity, motor or vocal responses to mechanical stimuli, or motor or vocal responses to a thermal stimulus.

To ensure naltrexone was blocking the antinociceptive properties of morphine, thermal reactivity was assessed following each training session. Animals in the two conditions (naltrexone or saline) did not have different latencies to exhibit a tail flick or vocalize in response to the thermal stimulus following saline treatment on either day. As shown in Figure 10, however, when given morphine animals treated with naltrexone

showed significantly lower response latencies than animals given saline (motor: $F_{(1,10)}=55.76$, $p<.05$; vocalizations: $F_{(1,10)}=5.37$, $p<.05$). This indicates the naltrexone was blocking the antinociceptive properties of the systemic morphine.

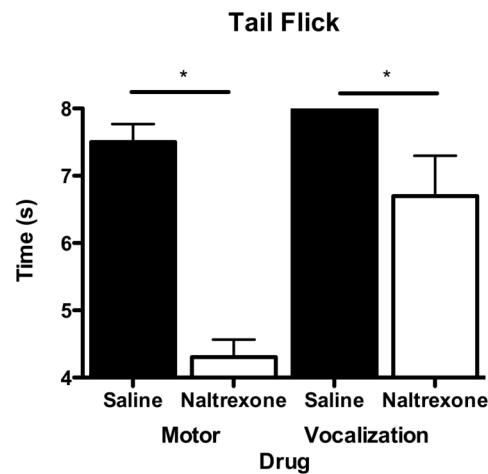


Figure 10. Following training, we tested the efficacy of naltrexone in blocking the antinociceptive properties of morphine. Animals given naltrexone had tail flick latencies that were significantly lower than animals given morphine alone. * $p<.05$

Assessment of Place-Preference. When looking at the preference for the morphine-paired context, there were no differences between the groups ($F_{(1,14)}<1.0$, $p>.05$; Figure 11).

Assessment of Sensory Reactivity After Place Preference Training. When place preference training and testing were complete, there were no differences between the groups on girdle, mechanical, or thermal reactivity (all p 's $>.05$). In addition, the

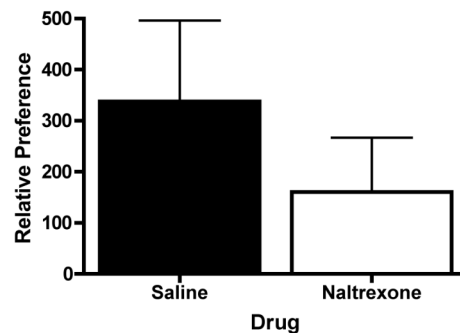


Figure 11. The assessment of conditioned place preference for the morphine-paired context revealed no differences between animals receiving i.t. saline versus i.t. naltrexone.

efficacy of naltrexone was confirmed. As shown in Figure 12, animals given naltrexone prior to morphine showed tail flick latencies comparable to those of animals treated with saline alone, and significantly lower than animals given vehicle and morphine (motor: $F_{(1,10)}=6.14$, vocalization: $F_{(1,10)}=5.98$, $p's < .05$).

Discussion

We found that i.t. naltrexone did not block the development of a morphine-induced place preference when the morphine was delivered systemically. The tendency for attenuated place preference when morphine was co-administered with i.t. naltrexone was not significant. This suggests that spinal loci may not be necessary for the induction of CPP after SCI. It seems changes at supraspinal loci may contribute to this preference for the morphine-paired context after SCI.

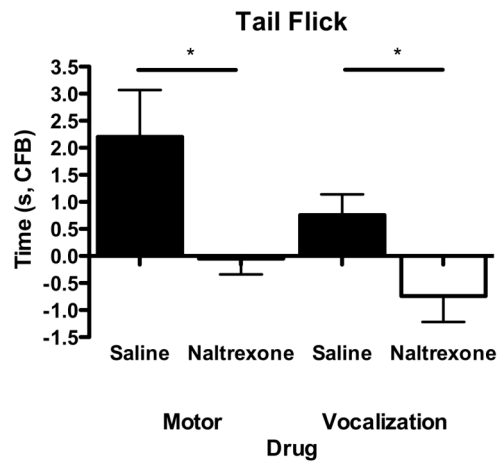


Figure 12. As found following training, animals given morphine and naltrexone following testing had tail flick latencies that were significantly lower than animals given morphine alone. * $p < .05$

EXPERIMENT 4

Experiment 3 suggests that spinal processes are not necessary for the development of a CPP. These findings suggest that a place preference for morphine may develop in the absence of analgesia. Nonetheless, the results do not exclude the idea that the place preference seen in the acute phase of SCI depends in large part on the antinociceptive properties of morphine. Morphine applied directly to the spinal cord, and producing antinociception, not reward, may be sufficient for expression of a CPP after SCI. To test this, Experiment 4 examined whether a preference for morphine would develop with an intrathecal administration.

Procedure

This experiment used 18 subjects (n=6). Animals received a moderate contusion injury, and were conditioned on the same timeline as that presented in Experiment 1 (see Figure 2). Rather than i.p. injections of morphine, however, subjects received an intrathecal (i.t.) injection of morphine (10, 30, or 90 μg) or vehicle. Morphine was delivered in 2 μl of distilled water and followed by a 20 μl injection of 0.90% saline to flush the catheter. The catheter was placed 2 cm caudal to the injury site to minimize supraspinally-mediated effects.

Results

Baseline Measures of Motor and Sensory Reactivity. BBB scores did not differ across groups on Day 1 following surgery ($F_{(2,15)}=2.05$, $p>.05$). Subjects had mean converted BBB scores ($\pm\text{SEM}$) of 2.00 ± 0.51 in the 10 μg group, 2.58 ± 0.46 in the 30 μg

group, and 3.25 ± 0.63 in the 90 μg morphine group. All scores were, again, indicative of a moderate contusion injury.

Similarly, there were no group differences on the tests of girdle or tactile reactivity and no differences in motor responses to the radiant heat stimulus (all p 's $> .05$). There was, however, a significant effect of assigned Dose on vocal responses ($F_{(2,15)}=3.69$, $p < .05$). *Post hoc* analysis revealed that subjects assigned to the 10 μg group vocalized later than those assigned to the 90 μg group ($p < .05$).

Assessment of Place Preference. As can be seen in Figure 13, there was a significant main effect of drug Dose on place preference ($F_{(2,15)}=4.99$, $p < .05$). Rats treated with 30 μg of morphine showed a preference for the drug-paired context, while those in the 10 μg and 90 μg morphine conditions did not.

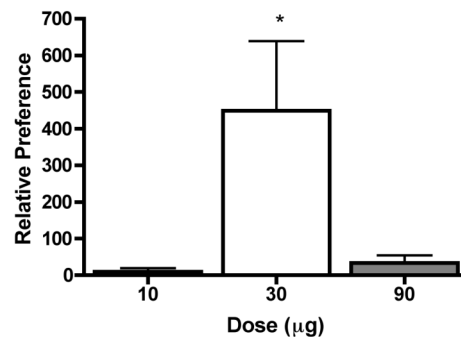


Figure 13. When the CPP was assessed, we found a significant preference for the morphine-paired context in the 30, but not the 10 or 90 μg groups. * $p < .05$

Assessment of Sensory Reactivity After Place Preference Training. Following place preference training and testing, there were no differences in girdle, mechanical, or thermal reactivity when the data were analyzed as a change from baseline. All doses of morphine provided the same level of antinociception (motor: $F_{(2,15)} < 1.0$, vocalization: $F_{(2,15)} = 1.84$, $p > .05$).

Discussion

We found that the delivery of 30 μ g morphine directly onto the spinal cord, in the acute phase of injury, results in a significant preference for the morphine-paired context. The morphine was delivered caudal to the site of injury to minimize supraspinally-mediated effects, suggesting that the preference is developing for analgesia rather than addiction. Taken together, the results of Experiments 3 and 4 suggest that spinal neurons are sufficient, but not necessary for acquiring a CPP.

CONCLUSIONS

These results suggest that a spinal contusion significantly increases the preference for a morphine-paired context in the acute, but not the chronic, phase of injury. Two days after injury, subjects showed an increased preference for the morphine-paired context relative to intact and sham controls. By contrast, subjects exposed to the morphine-paired context 14 days following injury failed to develop a preference. These data are commensurate with previous studies that report a decrease in the addictive potential of morphine after the development of neuropathic pain (Suzuki et al. 1996; Ozaki et al. 2002; Martin et al. 2007).

Pain experienced in the acute phase of injury differs substantially from pain experienced in the chronic phase, and these differences may be contributing to the development of a conditioned place preference. Whereas a place preference for morphine consistently emerged in the acute phase of injury with just two training trials, and occurred despite the use of low doses of morphine, a preference was not seen in animals experiencing neuropathic pain. Immediately following injury, animals are experiencing nociceptive pain as a result of the surgery, and this type of pain responds well to analgesics, such as morphine. In the chronic phase (14+days), however, most animals will have developed neuropathic pain (Mills et al. 2001). Indeed, in the present study, contused subjects showed increased girdle reactivity relative to sham and intact controls 14 days following injury. This is indicative of the development of at-level neuropathic pain. Morphine is considered to be one of the most effective treatments for neuropathic pain, but in the clinic this type of pain remains difficult to treat and often

involves the use of higher doses of opiates (Warms et al. 2002; Ozaki et al. 2003; Ballantyne & Mao 2003; Widerstrom-Noga & Turk 2003; Ozaki et al. 2002; Narita et al. 2004a).

Interestingly, in the current study subjects with symptoms of neuropathic pain did not appear to need a higher dose of morphine for analgesia. We found that 2.5mg/kg of morphine provided antinociception, as measured by the tail flick test, even in the chronic phase of injury. These data appear to be in contrast to clinical reports of unsatisfactory analgesia with high doses of morphine (Ballantyne & Mao 2003), but they concur with other animal studies suggesting that the antinociceptive efficacy of morphine is not reduced when the subject is experiencing neuropathic pain (Ozaki et al. 2003). Similar effects have also been reported for oxycodone, a semisynthetic opioid analgesic and μ -opioid receptor (MOR) agonist. Narita et al. (2008) demonstrated that while oxycodone did not produce a place preference, the amount needed for antinociception appeared to be comparable under conditions of neuropathic pain, and anti-inflammatory pain. The differences between the human and animal studies may be related to the behavioral assessment tasks used to document analgesia in the empirical experiments. In fact, the tail flick test that was used in each of the aforementioned studies, may not be an adequate measure of relief from neuropathic pain. In order to determine whether 2.5 mg/kg of morphine was providing adequate relief from at-level neuropathic pain assessed with a girdle test, a measure of girdle reactivity before and after morphine administration is needed. Unfortunately, however, we did not reassess girdle reactivity following morphine administration in the chronic phase of injury. It is

possible, therefore, that the lack of place preference seen in the chronic phase of injury was due to the reduced analgesic efficacy of low doses of morphine.

Alternatively, the lack of place preference observed after the development of neuropathic pain symptoms may be due to molecular modifications at a spinal level that undermine the rewarding properties of the drug after injury. Narita et al. (2004c) suggest that the activation of protein kinase C (PKC), resulting from a sciatic nerve ligation leads to the development of neuropathic pain, and is responsible for the attenuation of a morphine-induced place preference. It is thought that PKC activation causes the desensitization of μ -opioid receptors (MOR's), which leads to their dysfunction (Narita et al. 2007). In support of this, Narita et al. (2004c) have shown that administration of a PKC inhibitor, after sciatic nerve injury, re-instates the potential for the development of a conditioned place preference. In addition, it has been demonstrated in intact rats that intrathecal administration of a PKC activator leads to the development of hyperalgesia and an attenuation of a morphine-induced CPP; symptoms that are reversed when a PKC inhibitor is administered (Oe et al. 2004). These data suggest that PKC activation at the spinal level may be playing a role in the suppression of morphine-induced CPP after neuropathic pain has developed.

Furthermore, it is suggested that the activation of PKC may actually be causing changes in the endogenous opioid system (Niikura et al. 2008a&b), leading to attenuation of the rewarding properties of morphine in the chronic phase of injury. Specifically, sciatic nerve ligation has been found to increase the phosphorylation of the μ -opioid receptor in the spinal cord, thus decreasing the activity of the receptor (Narita

et al. 2004b). It has also been demonstrated that PKC, an activator of MAPK/ERK, leads to the release of the endogenous MOR ligand, β -endorphin, at the supraspinal level (Jin et al. 2003; Ji et al. 2003; Niikura et al. 2008a&b), which may lead to a decrease in MOR function in the ventral tegmental area (VTA). The VTA contains a high density of MOR's, and, along with the nucleus accumbens (N.Acc.), is critical for the reinforcing effects of morphine. μ -Opioid receptor desensitization is thought to occur, specifically, through an inhibition of the mitogen-activated protein kinase (MAPK) pathway (Polakiewicz et al. 1998). Moreover, a decrease in extracellular signal-regulated kinase (ERK) activity (Ozaki et al. 2004; Narita et al. 2004c) has been shown to result in the suppression of morphine-induced release of dopamine (DA) in the N.Acc after sciatic nerve injury (Narita et al. 2004a). So, in addition to the desensitization of MOR's, a decrease in DA release leads to the suppression of the rewarding properties of morphine. These data suggest neuropathic pain conditions cause multiple changes at spinal and supraspinal sites that affect the rewarding properties of morphine.

Neuropathic pain, however, has not developed in the acute phase of SCI. The CPP expressed in this phase may be largely due to the antinociceptive rather than hedonic properties of morphine. This idea is supported by the finding of a CPP with intrathecal morphine administration. Interestingly, however, even though all doses of i.t. morphine produced antinociception in Experiment 4, we only saw the development of a morphine-induced place preference for the 30 μ g dose. Again, it appears that the antinociceptive efficacy of morphine may, in fact, depend on the experimental test used to measure pain reactivity. Whereas 10 μ g of i.t. morphine effectively produces

antinociception as measured with the spinally-mediated tail flick task (Yu et al. 1997b), it is not typically effective in the relief of supraspinally-mediated mechanical allodynia in rodents (Lee et al. 1995; Yaksh & Harty 1987; Ossipov et al. 1995). In our experiments, we only used the tail flick test as an indice of analgesia, and this may not have been a comprehensive measure of pain relief. In fact, other studies have shown that 30 μg of morphine is the smallest dose that is effective in treating pain (Zhao et al. 2004; Hook et al. 2009). Furthermore, 90 μg of morphine can have aversive effects not related to analgesia (Chang et al. 2007; Yaksh & Harty 1987), which may be contributing to the lack of preference in this group. It has been demonstrated that high doses of i.t. morphine actually cause hyperalgesia (Woolf 1981; Yaksh & Harty 1987). Decreased analgesic effects, and the potential production of paradoxical pain, may have undermined the development of a CPP for the 10 and 90 μg doses, respectively. Overall, the finding that intrathecal morphine can mediate the development of a conditioned place preference after injury supports the hypothesis that this increased preference in the acute phase of injury is based on the antinociceptive effects of the drug.

The development of the preference for the antinociceptive effects of the drug may involve both central and peripheral opioid receptors. In Experiment 3, we did not find a significant difference in the development of a CPP when administering intrathecal saline or naltrexone prior to a systemic injection of morphine. Mu-opioid receptors exist both centrally and peripherally, and evidence suggests that peripheral MOR's can be important in alleviating persistent pain (Guan et al. 2008). In the current experiments, systemic morphine may still be acting on the peripheral MOR's to cause analgesia, while

central (spinal) MOR's are blocked by naltrexone, leading to the apparent, but not significant, reduction in preference. Central MOR's are typically considered the primary site of action for systemic morphine, leading to antinociception, but the peripheral MOR's may play a greater role when chronic pain is a factor (Guan et al. 2008). While these studies were done in the acute phase of injury, when nociceptive pain is present, the changes leading to the chronic phase of injury may be developing, leading to an increased role of the peripheral MOR's. It could also be that, in the acute phase of injury and before neuropathic pain has developed, the supraspinal reward systems are still intact and are contributing to the development of the preference. Further research is needed to determine the roles of spinal, peripheral, and supraspinal systems in the development of a CPP following injury.

Overall, these studies suggest that SCI itself does not reduce the addictive potential of morphine, but addiction may be reduced by neuropathic pain. Morphine is one of the most effective treatments for neuropathic pain, but concerns of dependence and addiction surround its use. The data reported here indicate that morphine could be used for the clinical treatment of neuropathic pain without concerns of addiction or psychological dependence. However, further research is needed to distinguish between the analgesic and addictive properties of morphine. In these studies, we used very low doses of morphine and an acute administration. These results may be quite different with chronic administration, and with use of higher doses of morphine as analgesic tolerance begins to develop. Overall, studies of place preference with the rodent contusion injury provides a tractable translational model to further examine changes in

the addictive potential of morphine, and other opiates, as neuropathic pain develops.

The contusion injury also provides a model system for the investigation of molecular changes that could reduce the potential for addiction, and even facilitate withdrawal in rehabilitation centers.

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