Morphine Self-Administration following Spinal Cord Injury

Sarah A. Woller,^{1,2} Jamal S. Malik,² Miriam Aceves,^{1,2} and Michelle A. Hook^{1,2}

Abstract

Neuropathic pain develops in up to two-thirds of people following spinal cord injury (SCI). Opioids are among the most effective treatments for this pain and are commonly prescribed. There is concern surrounding the use of these analgesics, however, because use is often associated with the development of addiction. Previous data suggests that this concern may not be relevant in the presence of neuropathic pain. Yet, despite the common prescription of opioids for the treatment of SCI-related pain, there has been only one previous study examining the addictive potential of morphine following spinal injury. To address this, the present study used a self-administration paradigm to examine the addictive potential of morphine in a rodent model of SCI.

Animals were placed into self-administration chambers 24 h, 14 d, or 35 d following a moderate spinal contusion injury. They were placed into the chambers for seven 12-hour sessions with access to 1.5 mg morphine/lever depression (up to 30 mg/d). In the acute phase of SCI, contused animals self-administered significantly less morphine than their sham counterparts, as previously shown. However, contused animals showing signs of neuropathic pain did not self-administer less morphine than their sham counterparts when administration began 14 or 35 d after injury. Instead, these animals administered nearly the full amount of morphine available each session. This amount of morphine did not affect recovery of locomotor function but did cause significant weight loss. We suggest caution is warranted when prescribing opioids for the treatment of neuropathic pain resulting from SCI, as the addictive potential is not reduced in this model.

Key words: addiction; morphine; neuropathic pain; self-administration; spinal cord injury

Introduction

IT HAS BEEN ESTIMATED that following spinal cord injury (SCI), 11–94% of people will experience pain, not only as a result of the injury itself but also from peripheral injuries sustained in unison, as in the case of motor vehicle accident.^{1–8} This pain is known to develop into chronic, pathological pain in approximately two-thirds of the spinally injured.² Not surprisingly, relief of chronic pain is a high priority to individuals with a SCI. In fact, when a quadriplegic population was surveyed, 15–20% indicated that the relief of chronic pain would most improve their quality of life.⁹

Although a considerable amount of research has focused on preventing the development of pain and developing effective therapies, it remains difficult to treat.¹⁰ Currently, chronic pain is treated with a variety of pharmaceuticals including opioids, antidepressants, anti-convulsants (e.g., gabapentin), non-steroidal anti-inflammatory drugs (NSAIDs), and N-methyl-D-aspartate (NMDA) receptor antagonists.^{11–15} While all show varying efficacy, opioids have long been considered a standard treatment in pain relief. Indeed, opioids are among the most effective treatments for neuropathic pain and are commonly trialed for analgesic efficacy.^{16–20}

In fact, until recently, opioid analgesics were considered to be a first-line medication for the treatment of neuropathic pain. Now, these medications are considered to be a second-line treatment.^{21,22} This change is the result of concern surrounding the presence of adverse effects, a lack of evidence demonstrating long-term safety of use, evidence showing continuous use is often associated with the development of dependence, tolerance, opioid-induced hyperalgesia, and/or addiction, and a lack of placebo-controlled trials assessing the efficacy of opioid treatment following SCI.^{18,23–27} It is known that, in the long term, approximately 20% of people will discontinue opioid treatment because of significant adverse effects such as respiratory depression, constipation, nausea, vomiting, sedation, dizziness, and headache.^{12,23,28,29} Notably, however, 80% of patients will continue to use opioids, leaving them vulnerable to the potential development of addiction.

While the addictive nature of opioid analgesics is well documented in a clinical setting, experimental data suggests that the abuse liability is reduced when opioids are used for the treatment of neuropathic pain.^{30–32} Both conditioned place preference and self-administration paradigms have been used to assess changes in the rewarding properties of these drugs.^{33–35} Using the conditioned place preference paradigm, Niikura and colleagues found that

¹Texas A&M University Institute for Neuroscience, ²Department of Neuroscience and Experimental Therapeutics, Texas A&M Health Science Center, Bryan, Texas.

animals experiencing pain resulting from a nerve injury do not develop a preference for a morphine-paired context.^{32,36,37} Lyness and colleagues also found that animals experiencing pain resulting from arthritis self-administered significantly less morphine.³⁸ Similarly, Martin and colleagues found that only doses of opioid that reverse mechanical allodynia maintain self-administration after a spinal nerve ligation, while lower doses maintained self-administration in sham-operated animals.³⁰ This suggests that animals are administering opioids to reverse pain but do not develop addiction-like behavior (like sham controls) when non-analgesic doses are used. These data suggest that the presence of neuropathic pain decreases the rewarding property of morphine.

Despite the common prescription of opioids for the treatment of SCI-related pain, however, there has been only one study exploring the addictive potential of morphine in an animal model of SCI. This study demonstrated that the intake of morphine varies depending on the experimental paradigm (conditioned place preference or self-administration) and the dose of morphine administered.³⁴ Contused animals given access to a moderate dose of morphine (1.5 mg/lever depression) self-administered significantly less morphine than their sham counterparts. When given access to a higher concentration (3.0 mg/lever depression), however, contused animals increased the amount of morphine administered over sessions to self-administer nearly the full 30 mg available. Moreover, administration of high amounts of morphine (more than 20 mg per session) resulted in an attenuation of locomotor recovery. Also, we have previously shown that a single dose of intrathecal morphine on the day following SCI undermined long-term recovery of locomotor function.^{39,40} These data suggest that there is a rightward shift in the addictive potential of morphine in the acute phase of SCI, and that high doses of morphine in this phase of injury have detrimental effects on long-term recovery of function.

These initial studies, however, did not address the effects of neuropathic pain on self-administration of opioids. The current study, therefore, aimed to test the hypothesis that neuropathic pain alters the administration of morphine. In the rodent contusion model, symptoms of neuropathic pain do not begin to emerge until 14 d post-injury and by 35 d post-injury, approximately 80% of subjects will show signs of pain.⁴¹ Thus, we compared self-administration in sham and contused subjects during the acute (Days 1–7), early chronic (Days 14–21), and chronic (Days 35–42) phases of injury.

Methods

Subjects

Male Sprague-Dawley rats obtained from Harlan (Houston, TX) were used as subjects. Animals were 90–110 d old (350–400 g), and were individually housed in Plexiglas bins (45.70 [length] × 23.50 [width] × 20.30 [height] cm) with food and water available *ad libitum*. Subject's bladders were expressed manually in the morning (8:00–9:30 AM) and evening (6:00–7:30 PM) 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-h light-dark cycle.

All of the experiments reported here were reviewed and approved by the Institutional Animal Care and Use Committee at Texas A&M and all National Institutes of Health guidelines for the care and use of animal subjects were followed.

Surgery

Jugular catheterization. To allow for the self-administration of morphine, animals were implanted with a jugular catheter as described previously by Woller and colleagues.³⁴ Rats were anesthetized

using a combination of 80 mg/kg ketamine and 10 mg/kg xylazine intraperitoneally (IP). While under anesthesia, a catheter consisting of a length of PE50 tubing was inserted into and tied to the jugular vein. Using an 11-gauge stainless steel tube as a guide, this catheter was passed subcutaneously through the body of the animal so that it exited in the back. A back mount cannula pedestal (model 313-00BM-10-SPC; Plastics One Inc., Roanoke, VA) was then implanted subcutaneously and connected to the catheter. The back mount exited the skin between the scapulae and was covered with a dust cap. All incisions were closed using VetBond.

For the first 24 h after surgery, the rats were housed in a recovery room maintained at 26.6°C. The subjects were treated with 100,000 U/kg penicillin G potassium IP immediately after surgery and again 2 d later. To help maintain hydration, the subjects also were given 3.0 mL of saline (0.9%, IP) following surgery. During the 5-d recovery period following surgery, the catheters were flushed with heparinized saline (0.25 mL).

Contusion injury. Five days after implantation of the jugular catheter, subjects were given a contusion injury as described by Hook and colleagues.³⁹ Briefly, 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. A 7-cm incision was made over the spinal cord, and two incisions extending 3 cm rostral and caudal to T12 were made on each side of the vertebral column. The lamina of the T12 vertebra was removed, exposing the spinal tissue. The vertebral column was then fixed within a Multicenter Animal Spinal Cord Injury Study impactor device.^{42,43} Animals were given a moderate contusion injury by allowing a 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.

For the first 24 h after surgery, the rats were housed in a recovery room as described previously. All subjects were treated with 100,000 U/kg penicillin G potassium (IP) immediately after surgery and again 2 d later. To help maintain hydration, the subjects also were given 3.0 mL of saline (0.9%, IP) following surgery. The Michel clips were removed 14 d following surgery.

Assessment of motor and sensory recovery

Locomotor recovery. Locomotor behavior was assessed using the Basso, Beattie, and Bresnahan (BBB) scale in an open enclosure (99 cm diameter, 23 cm deep) on the day following the contusion injury.⁴⁴ Subjects were acclimated to the apparatus for 5-min per day for 3 d prior to surgery. Twenty-four hours after surgery, each subject was placed in the open field and observed for 4 min to score locomotor function. All observers had high intra- and inter-observer reliability (all *r*'s > 0.89) and were blinded to the subject's experimental treatment.

Locomotor scores were transformed to help assure that the data were amenable to parametric analyses.⁴⁵ 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 obtained an ordered scale that is relatively continuous with units that have approximately equivalent interval spacing. Meeting these criteria allowed us to apply metric operations (computation of mean performance across legs), improve the justification for parametric statistical analyses, and increase 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 hind paws. Subjects were placed into Plexiglas tubes (7.00 cm [internal diameter] × 20.00 cm [length]) that had 6.00 cm (length) × 1.70 cm (width) notches removed from the

sides, to allow the hind limbs to hang freely. After a 15-min acclimation period, the von Frey stimuli were applied to the L5 plantar dermatome between the footpads sequentially at approximately 2 sec intervals until subjects withdrew the paw and vocalized.⁴⁶ If no response was observed, testing was terminated at an intensity of 6.65. Both a spinal (motor withdrawal) and supraspinal (vocalization to stimulation) measure of nociceptive reactivity were recorded. Testing continued until both a motor or vocal response was recorded or the maximal intensity of 6.65 was applied. 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: $\text{Intensity} = \log_{10}(10,000 \times \text{g force})$.

Girdle test. A measure of at-level neuropathic pain was derived using the girdle test, as described by Christensen and Hulsebosch.⁴⁷ For this task, 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 grid map of that animal. Girdle stimulation began at the left-rostral area and continued in a caudal direction until each of the 11 squares in that column was completed. The testing then began in the next column and was repeated until each of the 44 zones had been stimulated. For each subject, the total number of vocalizations was recorded.

Thermal reactivity. Thermal reactivity was assessed using radiant heat in the tail-flick test. Subjects were placed in clear Plexiglas tubes with their tail positioned in a 0.5 cm deep groove, cut into an aluminum block, and allowed to acclimate to the apparatus (IITC Inc., Life Science, CA) and testing room for 15 min. The testing room was maintained at 26.5°C. Thermal thresholds were then assessed. Thermal reactivity was tested using a halogen light that was focused onto the rat's tail. Prior to testing, the temperature of the light (focused on the tail) was set to elicit a baseline tail-flick response in 3–4 sec (average) in intact subjects. This preset temperature was maintained across all subjects. In testing, the latency to flick the tail away from the radiant heat source (light) was recorded. If a subject failed to respond, the test trial was automatically terminated after 8 s of heat exposure to prevent tissue damage. Two tests occurred at 2-minute intervals, and the second test tail-flick latencies were recorded as an index of spinal nociceptive reactivity. To confirm that subjects did not respond in the absence of the stimuli, blank trials were also performed. A "false alarm" was recorded if subjects made a motor or vocalization response

during the blank tests. The blank trials were performed 1 min before or after each sensory test (in a counterbalanced fashion). No false alarms were recorded.

Self-administration procedure

Apparatus. Self-Administration took place in operant chambers (Model E10-10 Coulbourn; Allentown, PA) enclosed in sound-attenuating cubicles. Each chamber was equipped with two levers with a stimulus light positioned over each. During the self-administration sessions, only depressions of the reinforced (right) lever were associated with morphine administration. The left lever was not reinforced. This distinction between the two levers allowed for the assessment of drug-seeking behavior, as animals will press the reinforced lever significantly more times than the non-reinforced lever if they find the drug to be rewarding. Animals in the control, saline, group do not learn an association and should not show increased responding on either lever.

Drug or saline delivery was controlled by infusion pumps (Razel Scientific Instruments; Stanford, CT) placed outside of the operant box. A 20-mL syringe was placed in each infusion pump and upon depression of the reinforced lever, advanced to deliver an intravenous infusion (160 μL) over a 6-sec time frame. The chambers were interfaced with two IBM computers, which controlled drug delivery and recorded lever depressions.

Self-administration procedure. In this experiment, animals were given a sham or contusion injury 5 d after the implantation of a jugular catheter. Twenty-four hours after the contusion injury, baseline tests of locomotor and sensory reactivity were conducted. Based on BBB scores, animals were divided into groups, with scores balanced across groups (Fig. 1). In one group, animals were placed into the self-administration chambers for seven nights beginning 24 h after injury. In the chambers, animals were given access to 0.0 or 1.5 mg morphine (National Institute of Drug Abuse, Bethesda, MD), up to 30 mg morphine/ session. In this early phase post-injury, animals show little to no movement in the hind limbs. This, however, does not affect the ability of the animal to perform the required response. In a previous publication, we showed that contused animals given access to 3.0 mg morphine self-administered the full amount available to them, while those given access to 1.5 mg per infusion did not administer the full amount available.³⁴ Animals in each group had equivalent BBB scores prior to the self-administration sessions, indicating that the differential responding across doses was not due to differences in motor recovery per se. In addition, animals in the 3.0 mg group would continue to press the

FIG. 1. Animals were implanted with a jugular catheter, and received a contusion or sham injury 5 d later. One group of rats was placed into the self-administration chambers 24 h following spinal cord injury (SCI) or the sham surgery, another group 14 d following SCI or the sham surgery, and a third group 35 d following SCI or the sham surgery. Each group had access to 1.5 mg morphine/ lever depression and up to 30 mg each session.

lever after the drug was exhausted, further indicating that they can perform the task without difficulty related to their SCI.

A second group of animals were placed into the self-administration chambers in the early chronic (Days 14–21) phase of injury. As with the acute group, these animals were placed into the self-administration chambers for 12 h during their dark cycle for seven nights. In the chambers, they were given access to 0.0 or 1.5 mg morphine, up to 30 mg morphine/session. Animals in the final group were placed in the chambers in the late chronic phase of injury (Days 35–42). These animals had access to 1.5 mg morphine, up to 30 mg/session, for seven sessions. The dose of morphine per lever press and total dose of morphine available was chosen based on previous studies. This dose of morphine per lever press significantly differentiated self-administration behavior in SCI and sham-injured subjects in the acute phase of spinal injury.³⁴ Woller and colleagues demonstrated that although sham subjects administered the total 30 mg available in the acute phase of injury, contused subjects only administered approximately 10 mg morphine when 1.5 mg was administered with each lever press.³⁴

Statistical analysis

Comparisons between the amounts of morphine administered across surgery, dose, and phase of injury, as well as the effects of morphine on recovery of function and molecular endpoints, were analyzed using analysis of variance (ANOVA) and trend analyses. In experiments with a continuous independent variable (e.g., recovery period), mixed-design ANOVAs were used. In cases where significant between-subject differences were obtained (main effect of a single variable), group means were compared using the Duncan's new multiple range test ($p < 0.05$).

Results

Morphine self-administration is decreased in the acute phase of spinal cord injury

Baseline sensory reactivity. Twenty-four hours following a contusion ($n = 12$; 6 morphine, 6 saline) or sham ($n = 12$; 6 morphine, 6 saline) injury, baseline sensory reactivity tests were conducted. These tests measured mechanical reactivity, thermal hyperalgesia, and the development of at-level neuropathic pain (girdle test). Using von Frey filaments to measure mechanical reactivity, we found that sham animals withdrew their hind limb at a lower threshold than contused animals ($F [1, 22] = 17.87, p < 0.05$; Fig. 2A). Vocalizations to the mechanical stimuli also were recorded. Here, sham animals vocalized at a lower threshold than contused animals but this result only approached significance ($F [1, 22] = 3.98, p = 0.059$; Fig. 2A). The reduced spinal-supraspinal communication in the acute phase of a contusion injury likely reduced reactivity to stimuli applied below the level of the lesion. While contused animals were less reactive for the measure of below-level mechanical reactivity, they vocalized more than sham animals on the test of at-level pain, girdle reactivity ($F [1, 21] = 6.93, p < 0.05$; Fig. 2B). No other results approached significance (F 's $< 1.2, p > 0.05$).

Morphine administered. These data replicate our previous studies demonstrating that self-administration behavior is attenuated in the acute phase of SCI.³⁴ Comparison of the number of lever presses made by subjects when morphine and saline was administered (drug-linked lever) across the 7 d of administration showed a main effect of surgery but not drug condition. Sham subjects made significantly more responses than contused subjects on the drug-linked lever ($F [1, 20] = 12.06, p < 0.005$; Fig. 3A). Sham subjects also made significantly more lever presses when the total number of presses, across the right and left levers, were compared

FIG. 2. Baseline tests of mechanical reactivity (A) and girdle reactivity (B) were conducted on the day following the contusion/sham surgery and prior to morphine administration. Contused animals were less reactive on motor tests of mechanical reactivity. However, contused rats vocalized more in response to mechanical stimuli applied at and above the level of injury. $*p < 0.05$

($F [1, 20] = 8.61, p < 0.01$). As can be seen in Figure 3B, this difference in responding likely emerged in the early phase (Days 1–3) of administration. Comparison of the percentage of total responses made on the drug-linked lever across groups showed a main effect of drug condition ($F [1, 16] = 4.51, p < 0.05$) and a significant surgery $\times$ drug condition interaction ($F [1, 16] = 6.73, p < 0.05$). Subjects administering morphine made a greater percentage of responses on the drug-linked lever, relative to subjects administering vehicle, and this preference was primarily driven by the contused subjects. For the sham subjects, both the vehicle and morphine-administering subjects made most of their response on the drug-linked lever (Fig. 3C).

Commensurate with the lever pressing behavior, contused subjects administered significantly less morphine (1.5 mg/lever press) than sham subjects in the acute phase of injury. Contused animals self-administered only 8.4 mg on average versus 21.1 mg in the sham group (Fig. 3D). An ANOVA revealed a main effect of surgery ($F [1, 10] = 15.43, p < 0.05$).

FIG. 3. (A) Sham subjects made significantly more responses on the drug-linked lever. (B) Similarly, sham subjects responded more when both the reinforced and non-reinforced responses were combined. (C) Both sham and contused subjects administering morphine showed a greater percentage of responses on the drug-linked lever, relative to saline. (D) Replicating previous studies, contused animals administered significantly less morphine than sham animals across the seven administration sessions when given access to a 1.5 mg per lever depression. $*p < 0.05$

Rate of administration. By deriving the amount of morphine administered in each of the 12 one-hour time bins comprising an administration session, we were able to examine how rapidly animals administered morphine (Fig. 4). As shown in Figure 4, even on Day 1, contused and sham rats diverge in the amount of morphine administered and in the amount of time spent pressing the lever. Across days, contused rats maintained a moderate and steady level of administration, administering morphine across the first 8 hours of the sessions (on Day 7). This low rate of drug administration is commensurate with the analgesic efficacy of the drug. In contrast, sham animals administered higher amounts of morphine and escalated in administration across days.

Locomotor recovery. Locomotor recovery was monitored for a 42-d period in both sham and contused subjects. Sham controls received a maximum converted BBB score of 12 on the day following surgery, and this remained unchanged throughout the period of recovery in both vehicle and morphine groups. Subsequent an-

alyses, therefore, focused on the recovery of the contused groups only. To investigate the effect of morphine administration on recovery of locomotor function, we separated the data into a period of administration (Days 1–7) and a period of recovery (Days 8–42). There was no significant effect of morphine administration on recovery of locomotor function at the dose administered (F 's < 1.0 , $p > 0.05$). Administering 10 mg/morphine per session or less, contused animals recovered to the same level of locomotor function as those administering saline (Fig. 5).

Weight. As with locomotor recovery, weight data was separated into a period of administration (Days 1–7) and a period of recovery (Days 8–42). During administration, sham and contused animals lost weight in both the 0.0 mg and 1.5 mg groups (Fig. 6A and Fig. 6B, respectively). Contused animals in the saline group lost more weight during this phase of injury. These results are supported by a day $\times$ drug condition interaction (F [6, 120] = 2.81, $p < 0.05$).

FIG. 4. The cumulative amount of morphine administered by hour on Day 1 (A), Day 3 (B), Day 5 (C), and Day 7 (D) is depicted. In each administration period, sham animals administered significantly more morphine than contused subjects when 1.5 mg/morphine was administered per lever press, administering 20–30 mg of morphine within 5–9 h. The contused animals administered a moderate amount of morphine (approximately 10 mg) over a 6- to 8-h period.

FIG. 5. Replicating previous results, a 1.5 mg infusion of morphine per lever press, and approximately 10 mg of morphine per day, did not affect recovery of locomotor function when administration was initiated in the acute phase of spinal cord injury (shaded area).

During the period of recovery (Days 8–42), contused animals maintained a lower weight than sham animals in both the 0.0 mg and 1.5 mg groups (Fig. 6A and Fig. 6B). Morphine administration did not significantly increase this weight loss in the contused groups. Weight loss in the sham animals was increased by morphine administration but this effect did not persist throughout the recovery period. There were significant main effects of drug condition ($F [1, 20] = 6.66, p < 0.05$) and surgery ($F [1, 20] = 11.48, p < 0.05$) on weight loss. As can be seen in Figure 6, morphine-treated animals lost more weight than saline controls, and the contusion produced greater weight loss than the sham injury. In addition, there were significant

day $\times$ drug condition ($F [11, 220] = 7.66, p < 0.05$), day $\times$ surgery ($F [11, 220] = 7.94, p < 0.05$), and day $\times$ drug condition $\times$ surgery ($F [11, 220] = 6.99, p < 0.05$) interactions. Post hoc tests indicated a significant difference between sham animals administering morphine and sham animals administering saline ($p < 0.05$).

Sensory reactivity. The tests of girdle, tail flick, and hind paw reactivity were repeated 42-d after the contusion or sham injury. At this time point, there was no lasting effect of previous morphine administration (Days 1–7) on mechanical reactivity, thermal hyperalgesia, or girdle reactivity (All F 's $< 3.84, p > 0.05$). However,

FIG. 6. Both contused and sham animals lost weight during the period of self-administration (shaded area). Contused animals (**B**) lost more weight than sham animals (**A**). While morphine administration did not increase weight loss during the period of administration, it attenuated the rate of weight gain across the recovery period.

FIG. 7. This figure depicts the latency to flick the tail away from a noxious heat stimulus in the chronic phase of injury and 35 d following morphine administration. At 42 d following injury, contused animals showed a significant decrease in tail flick latency, relative to sham controls. There was no effect of morphine administration on this measure of spinal reactivity. $*p < 0.05$

a contusion injury significantly decreased tail flick latency regardless of drug treatment ($F [1, 1] = 8.65$, $p < 0.05$; Fig. 7). No other results approached significance (F 's < 4.1 , $p > 0.05$).

Morphine self-administration is not decreased in the early chronic phase of injury

In the acute phase of injury, we observed a decrease in morphine self-administration in injured animals. These animals, however, were not yet experiencing neuropathic pain. The idea that neuropathic pain reduces the abuse liability of opioids has not been examined following a spinal cord injury. Thus, we assessed morphine intake in the chronic phase of SCI (after symptoms of neuropathic pain have developed) using a self-administration paradigm.

Sensory reactivity. In this group, baseline tests of sensory reactivity were conducted 14 days after injury, before morphine self-administration sessions began. At this time, contused animals ($n = 12$; 6 morphine, 6 saline) withdrew their hind limbs to a lower

FIG. 8. At 14 d post-surgery and prior to the initiation of morphine self-administration, contused animals displayed signs of mechanical allodynia, compared with sham controls. $*p < 0.05$

force von Frey filament than sham animals ($n = 12$; 6 morphine, 6 saline), indicating these animals are showing signs of increased mechanical reactivity. There was a main effect of surgery on mechanical reactivity thresholds ($F [1, 22] = 13.84$, $p < 0.05$; Fig. 8). There were no differences in vocalization to a mechanical stimulus, thermal hyperalgesia, or response to girdle stimulation (all F 's < 1.0 , $p > 0.05$).

Morphine administered. Comparison of the number of presses of the drug-linked lever between contused and sham animals administering morphine and saline in the chronic phase of injury revealed significant main effects of both surgery ($F [1, 19] = 8.47$, $p < 0.01$) and drug condition ($F [1, 19] = 7.36$, $p < 0.05$) but no interaction between variables. Contused subjects made significantly more lever presses than sham subjects, and subjects administering 1.5 mg morphine pressed the drug-linked lever significantly more than subjects administering saline (Fig. 9A). Contused subjects administering morphine persisted in pressing the drug-linked lever even when the drug was no longer available (exceeding 20 lever presses on the drug-linked lever). For total lever presses, cumulating responses made on both the left and right levers, there was no effect of surgery or drug condition. Comparison of the percentage of total responses made on the drug-linked lever across groups showed a main effect of drug condition ($F [1, 19] = 5.41$, $p < 0.05$), with no effect of surgery or surgery $\times$ drug condition interaction. Subjects administering morphine made a greater percentage of responses on the drug-linked lever, relative to subjects administering vehicle, irrespective of the injury condition (Fig. 9C).

Further, in contrast to the acute phase of injury, we found contused and sham animals took similar amounts of morphine when administration began 14 d following injury ($F < 1.0$, $p > 0.05$). On average, injured animals self-administered 25.61 mg/d while sham rats self-administered 19.86 mg/d (Fig. 9D).

Rate of administration. In the early chronic phase of injury, the rate of administration in the contused rats was different than the acute phase. On Day 1 of administration, contused animals administered an amount of morphine commensurate with sham controls. They administered around 20 mg of morphine in the first 5 h of the self-administration session (Fig. 10). The amount they administered increased over days, and by Day 7 they were administering nearly the entire 30 mg. This administration occurred over 10 h of the session. While the amount they administered was greatly increased, it was administered over a longer period of time (Fig. 10).

Locomotor recovery. In this experiment, the 42 d were separated into three periods: prior to morphine administration (Days 1–13), during morphine administration (Days 14–20), and during recovery (Days 21–42). Despite administration of a large amount of morphine, there was no effect of morphine on recovery of locomotor function in any of these periods (all F 's < 1.0 , $p > 0.05$; Fig. 11).

Weight. As with recovery of locomotor function, weight change was examined in three periods. Prior to morphine administration (Days 1–13), contused animals lost weight relative to sham controls (Fig. 12A and Fig. 12B). This result was supported by a significant main effect of surgery ($F [1, 20] = 54.75$, $p < .0001$) and a day $\times$ surgery interaction ($F [9, 180] = 13.39$, $p < 0.0001$).

During the period of morphine administration, contused rats continued to weigh less than sham animals and weight loss was increased by the administration of morphine. There was a significant main effect of surgery ($F [1, 20] = 26.52$, $p < 0.0001$), and a

FIG. 9. (A) In the early chronic phase (Days 14–21), contused subjects made significantly more responses on the drug-linked lever than sham animals and those administering saline. (B) However, there was no effect of drug condition or surgery on the total number of lever presses. (C) Subjects administering morphine made a greater percentage of presses on the drug-linked lever. (D) Sham and contused rats self-administered equal amounts of morphine.

day $\times$ drug condition interaction ($F [1, 20] = 9.41, p < 0.01$) for weight loss in this period. As can be seen in Figure 12A and B, contused rats administering morphine weighed significantly less than injured subjects treated with saline or sham subjects.

During recovery (Days 21–42), all rats began to regain weight but contused rats remained smaller than their sham counterparts. In addition, both contused and sham animals administering morphine weighed less than injured and sham animals administering saline. Supporting these results, an ANOVA yielded a significant main effect of drug condition ($F [1, 20] = 10.64, p < 0.01$), and surgery ($F [1, 20] = 16.99, p < 0.001$), and a significant day $\times$ drug condition interaction ($F [6, 120] = 20.918, p < 0.0001$). As found previously, morphine administration increased weight loss as did the contusion injury, relative to sham. Post hoc analyses indicated that contused animals administering morphine lost significantly more weight than the contused animals administering saline ($p > 0.05$), weighing only 4.86 grams more on Day 42 than on Day 1, versus 22.41 grams more in the saline group.

Sensory reactivity. Forty-two days after injury, there was no lasting effect of previous morphine administration (Days 14–21) on

measures of sensory reactivity (all F 's $< 1, p > 0.05$). A contusion injury significantly increased vocalizations on the girdle test of at-level neuropathic pain ($F [1, 1] = 5.75, p < 0.05$; Fig. 13). No other results approached significance (all F 's $< 1.1, p > 0.05$).

Contused rats showing established neuropathic pain do not self-administer less morphine

The results for the early chronic phase are in contrast to those in the literature reporting a reduced morphine reward in animals with neuropathic pain resulting from a peripheral injury. In the contusion model of SCI, symptoms of neuropathic pain typically begin to develop in a subset of animals (approximately two-thirds), around 14 d post-injury.^{47–49} However, in our study, many of the animals were not showing signs of neuropathic pain at this early time point. Thus, we extended the period of administration to begin 35 d after injury, when neuropathic pain is more established. Here, we examined only the amount of morphine administered in contused and sham animals given access to 1.5 mg of morphine and their sensory reactivity. As there was no effect of morphine administration on locomotor recovery in the early chronic phase of SCI, we did not monitor recovery in these animals.

FIG. 10. The cumulative amount of morphine administered across the 12-h self-administration sessions from Days 14–21 is depicted. In this early chronic phase of spinal cord injury, contused animals administered significantly more morphine than contused animals in the acute phase, administering an amount commensurate with sham controls. Contused and sham subjects administered up to 30 mg of morphine within 7 h of initiation of the 12-h administration session.

FIG. 11. The locomotor recovery of contused subjects administering morphine or vehicle is shown. Despite administering 20–30 mg of morphine per day, there was no effect of morphine administration on recovery of locomotor function in the chronic phase of injury.

Sensory reactivity. Baseline measures of sensory reactivity were recorded on Day 35 following injury, before initiation of self-administration sessions. In a test of mechanical allodynia, contused subjects ($n=12$; 6 morphine; 6 saline) withdrew their paw at a higher threshold than sham animals ($n=12$; 6 morphine; 6 saline; $F[1, 10]=7.65$, $p<0.05$) but were not different in vocalizations ($F<1.0$, $p>0.05$). At this time, however, we found contused animals showed shorter tail flick latencies than sham animals, indicating the development of thermal hyperalgesia. This result approached significance ($F[1, 10]=3.84$, $p=0.07$). In addition, we found contused animals vocalized more in the girdle test than sham animals (an average of 10.2 vocalizations vs. 1.0, respectively),

indicating the development of neuropathic pain. Commensurate with the literature, this increase was seen in 66% of our contused animals. Due to the small number of subjects, this increase in vocalizations, compared with sham controls, was not significant ($F<1.0$, $p>0.05$).

Morphine administered. As in the early chronic phase, contused animals administered as much morphine (21.5 mg/d average) as sham controls (22.9 mg/d average), shown in Figure 14. An ANOVA showed there was not a difference between these two groups ($F[1, 10]<1.0$, $p>0.05$).

Rate of administration. As in the early chronic phase, animals in this phase of injury began by administering approximately 25 mg of morphine in 6 h on Day 1 (Fig. 15). Rather than increasing the rate and amount administered over days, however, they remained consistent in the time they administered (6–7 h).

FIG. 12. Prior to morphine administration, contused animals (B) lost more weight than sham animals (A). During the period of administration, contused animals continued to lose weight, and morphine administration exacerbated weight loss. Contused subjects that self-administered morphine lost more weight than all other groups. During the recovery period, all rats regained weight but contused animals failed to regain weight at a rate commensurate with sham controls. In addition, contused rats administering morphine weighed less than those administering saline.

Discussion

Replicating our previous experiments, injured animals self-administered significantly less morphine than their sham counterparts in the acute phase of SCI. This decrease in administration suggests that there is a decreased abuse liability to morphine in the early phase of spinal injury. The current study, however, suggests that any protection from addiction does not extend into the chronic phase of injury. Subjects given access to morphine in the early

FIG. 13. Forty-two days following injury, contused animals vocalized significantly more on a test of at-level girdle reactivity. There was no effect of morphine treatment on this measure of at-level pain. $*p<0.05$

FIG. 14. The amount of morphine administered by contused and sham-injured animals from Days 35–42 is shown. In this late chronic phase of injury, contused animals administered as much morphine as sham animals.

chronic and chronic phases of SCI administered high amounts of morphine at a rate commensurate with sham controls. Overall, our results demonstrate that the intake of morphine varies depending on the phase of injury in which it is administered, and that neuropathic pain symptoms do not negate opioid reward in this model.

The present results are in contrast to reports of a reduced abuse liability of morphine in the presence of neuropathic pain in peripheral injury models. The variation in results between our study and peripheral models may stem from differences in experimental design (e.g., amount of morphine available, length of self-administration sessions, or prior experience in self-administration chambers). For example, Martin and colleagues found dose-dependent decreases in morphine self-administered following a spinal nerve ligation.³⁰ Animals experiencing pain self-administered less morphine at doses up to 180 μ g/kg but not at higher doses (up to 600 μ g/kg). The doses used in their study, compared with ours (and those used clinically) are low: 0.18 mg/kg versus approximately 5 mg/kg in our 1.5 mg group. While lower doses were not investigated in the current study, it is possible that contused animals would self-administer significantly less morphine than sham controls at lower doses. Indeed, in a previous study we found contused animals self-administered significantly less of the 1.5 mg dose (the dose used in the current study) in the acute phase of injury, compared with a 3.0 mg dose. This was replicated in the current study but interestingly, the reduced administration of morphine after SCI did not extend into the chronic phase of injury.

Our experimental design also differed in the length of the self-administration sessions. In studies of cocaine self-administration,

session length has been shown to be an important factor in determining both the rate (infusions/hour) and amount of cocaine self-administered.^{50,51} In a 12-h session, more cocaine was self-administered, relative to a one hour session, and was administered in a shorter amount of time.⁵¹ The Martin study used four 1-h sessions in which different doses of drug were available each hour.³⁰ In contrast, our studies have all used 12-h sessions. With a 12-h session, it is anticipated that the rats will self-administer morphine more rapidly than would be seen in a 1-h session. It is important to note that the 12-h session also more closely resembles patient controlled analgesia, making use of longer sessions clinically relevant in the study of addictive behavior.

With the 12-h session allowing for the assessment of addictive behavior, we found contused animals administering the 1.5 mg dose in the acute phase of injury did not display high levels of lever pressing.^{50,51} These contused animals maintained a moderate rate of morphine administration across the seven sessions—displaying a preference for the reinforced lever (Fig. 3C) but not administering an excessive amount of morphine within sessions. While there was a significant effect of surgery on lever-pressing behavior, this effect appeared to be largely due to reduced pressing in the early stage of administration. Indeed, as noted previously, the infusion of a higher dose of drug per lever press (3.0 mg) is sufficient to increase lever pressing in the acute phase of injury, with contused subjects administering almost 30 mg of morphine per administration session from Days 4–7 post-injury.³⁴ The reduced lever pressing behavior is not, therefore, simply due to an inability to press the lever per se. In contrast to the acute phase, in the chronic phase of injury, contused animals self-administered an amount of morphine that was commensurate with sham controls. Any protection from addiction appears to have abated by the chronic phase of injury as both the sham and SCI subjects display rapid administration of large amounts of morphine.

While contradictory to other models, a simple explanation for the increased morphine administration in the chronic phase of injury is the increased need for analgesia with the development of neuropathic pain. Indeed, up to 66% of our animals were showing signs of neuropathic pain in the chronic phase of SCI. Although self-administration behavior is typically used as an index of addiction, it must be acknowledged that the current experiments cannot delineate between administration for addiction and analgesia. In the chronic phase of injury, the SCI subjects may be administering large amounts of morphine to counter symptoms of neuropathic pain. Unfortunately, due to limitations of the self-administration sessions (varied amounts of morphine administered

FIG. 15. The cumulative amount of morphine administered across the 12-h administration sessions is depicted. As found at earlier stages of injury, contused and sham-injured subjects administered their total dose of morphine within 7–8 h of initiation of the administration sessions.

overall and the time in which it was administered, and time since the last infusion of morphine relative to the end of the session), we were not able to test analgesic efficacy of morphine throughout this experiment. This needs to be tested in future studies. Nonetheless, the doses administered (30 mg to an ~ 300 g rat; 100 mg/kg) and the short period of time (6–7 hrs) in which the morphine was administered equate to a dose of morphine that is far greater than that needed to sustain analgesia after SCI (20 mg/kg).⁵² This pattern of administration in the chronic phase of injury is suggestive of addiction.

Rather than analgesia, we hypothesize that the differential self-administration seen in the acute and chronic phases of SCI may be due to changes in the molecular mechanisms inherent to these specific stages of injury. For example, in the acute phase of SCI, increases in spinal dynorphin levels may activate spinal kappa-opioid receptors (KORs) to oppose morphine-induced analgesia.⁵³ Indeed, both SCI and morphine administration are known to increase spinal dynorphin levels.⁵⁴ In the absence of rewarding analgesic effects, contused subjects given access to the 1.5 mg dose may not be motivated to continue administering the drug. Supporting this, as discussed previously, Martin and colleagues found that only doses of morphine that reversed mechanical allodynia maintained self-administration after spinal nerve ligation.³⁰ Again, the analgesic efficacy of the 1.5 mg dose of morphine was not tested in the current study. However, we know from previous studies that 20 mg/kg systemic morphine (equivalent to two to three concurrent presses in the 1.5mg group) is sufficient to provide a robust analgesia in the acute phase of a spinal contusion injury.⁵² Further, the 1.5 mg of morphine administered is approximately 10-fold higher than the 600 μ g/kg used by Martin and colleagues to reverse allodynia following a spinal nerve ligation.³⁰ Because the amount of morphine contused animals self-administered far exceeds that needed for analgesia and the doses used have been shown to reverse mechanical allodynia in other models, it is unlikely that the attenuated self-administration observed in the contused subjects simply reflects a reduction in the analgesic efficacy of morphine.

In addition to increasing spinal levels of dynorphin, SCI has been shown to modulate supraspinal levels of preprodynorphin in the acute phase of injury.⁵³ It is possible that in the acute phase of SCI, an increased expression of dynorphin alters opioid reward.⁵⁵ As with spinal activation, increased activation of the KOR is thought to oppose reward at a supraspinal level.⁵⁶ For example, genetic differences in brain dynorphin levels have been shown to make various strains of mice less responsive to the rewarding effects of morphine.⁵⁷ Mouse strains with high endogenous levels of dynorphin will not develop a preference for a morphine-paired context at a low dose of morphine. When the KOR is antagonized with norbinaltorphimine, the same dose of morphine will produce a conditioned place preference.⁵⁷ Further, it is known that dynorphin binds to KOR in the nucleus accumbens to decrease dopamine levels, supporting the idea that the activation of KOR antagonizes reward, even at supraspinal sites.^{58–60} Further understanding of the relationships between SCI, dynorphin, and addiction is warranted.

In addition to differences in the self-administration of morphine in the acute and chronic phases of SCI, the effects of morphine on recovery appear to depend on the phase in which the drug is applied. Previously, we have shown that morphine administration in the acute phase of SCI significantly undermines recovery of locomotor function.³⁴ This effect, however, depends on the amount of morphine being administered. Animals administering on average 50 + mg/kg/d showed significantly impaired recovery, whereas those administering less recovered to the same level as saline controls.³⁴

Surprisingly, however, in the chronic phase of injury, all contused animals self-administered more than 50 mg/kg/d and did not show an attenuation of locomotor recovery. This suggests that the spinal circuits underlying locomotor recovery are more vulnerable to the effects of morphine administration in the acute, rather than in the chronic, phase.

We hypothesize that although dynorphin may attenuate the potential for addiction, it increases activation of the KOR to increase excitotoxicity and paralysis at the spinal level. In intact rats, increases in spinal dynorphin are associated with locomotor deficits, and spinal cord injury is known to cause an increase in dynorphin levels contributing to the secondary pathology of injury.^{61–66} Increases in dynorphin and preprodynorphin have been reported in spinal tissue as early as 2 hours post-injury with elevated levels observed up to two weeks post SCI.^{53,64,67,68} KOR binding also appears to peak at 24 h post-injury, decreasing over the following days, at the spinal injury site.⁶⁹ Further, Adjan and colleagues found that dynorphin, following a T10 contusion injury in mice, is associated with the expression of caspase-3 in more than 90% of all neurons, oligodendrocytes, and astrocytes.⁶⁸ We hypothesize that the synergistic effect of opioid administration and SCI in the acute phase of injury, when endogenous dynorphin levels and KOR binding are maximal, leads to the increased cell death and attenuation of recovery of locomotor function.⁷⁰ As endogenous dynorphin levels decrease in the more chronic stages of SCI, the administration of morphine alone may not be sufficient to potentiate cell death and decrease recovery of function.

While increased activation of the KOR may be one mechanism modulating recovery, proinflammatory cytokines (such as interleukin [IL]-1 β) have also been implicated in the morphine-induced attenuation of locomotor function.⁴⁰ Glial activation and the release of pro-inflammatory cytokines also are characteristic of the acute phase of SCI. Following SCI, astrocyte activation in the thoracic spinal dorsal horn peaks between 24 h to 7 d post-injury, and microglia activation is maximal at 24 h.⁷¹ Upon activation, glial (astrocytes and microglia) cells, rather than maintaining homeostasis, become dysfunctional and contribute to neuronal hyperexcitability.⁷² Morphine administration can further contribute to dysfunction through binding at TLR4. Upon activation of the TLR4, glial cells release pro-inflammatory cytokines (IL-1 β and TNF α) that contribute to inflammation and secondary damage.^{73–77} For the spinal contusion model, our previous studies have demonstrated that SCI increases the levels of IL-1 β in the acute phase of injury, and that morphine administration further increases the expression levels of IL-1 β .⁴⁰ Moreover, antagonizing the IL-1 receptor prior to morphine administration blocks the morphine-induced attenuation of locomotor function.⁴⁰ Activation of the IL-1 receptor is, therefore, necessary for the morphine-induced attenuation of recovery in the acute phase of SCI. It is noted that glial activation continues for up to 180 d following SCI, and increases in pro-inflammatory cytokines are seen up to 28 d but the levels are not as high as in the acute phase of SCI.⁷¹ Pro-inflammatory cytokine expression may not exceed a threshold for adverse effects in the chronic phase of injury. In addition, it is unlikely that any one mechanism inherent to the acute phase of SCI is causing deficits in locomotor function alone. Rather, synergistic effects of multiple mechanisms likely potentiate hyperexcitability and dysfunction in the acute phase of SCI. Overall, morphine administration in the chronic phase of SCI does not undermine locomotor recovery, whereas it does in the acute phase. From a clinical perspective, aside from concerns of addiction, morphine use for the treatment of pain in the chronic phase of injury should not affect recovery of locomotor function.

In these experiments, we also saw weight loss occurring as a result of morphine administration. Weight loss can result from withdrawal syndrome, which may contribute to the weight loss we observed in the early chronic phase of injury.⁷⁸ Indeed, the high amounts of morphine administered each day in the early chronic phase of injury led to signs of withdrawal (e.g. wet-dog shakes, teeth chewing) throughout the period of administration and into the recovery period, though these were not quantified. Despite this plausible explanation, we propose that the weight loss is not due solely to withdrawal or alterations in food/fluid consumption.^{79,80} Hook and colleagues have shown that a single intrathecal administration of morphine produces lasting weight loss in rats.^{39,40} This weight loss cannot result from withdrawal, and acute administration of morphine does not significantly affect food intake.⁸⁰ This result suggests that opioid administration is causing a dysregulation in the acute and chronic phases of injury leading to weight loss; however, the cause of this weight loss remains to be determined.

Overall, the data presented here suggest that the phase of injury critically affects the adverse outcomes associated with morphine administration. Nonetheless, the use of morphine may be fraught with adverse consequences, irrespective of the time of use. We have shown that morphine administered in the acute but not the chronic phase of injury attenuates recovery of locomotor function. Based on these findings, use of opioid analgesics, at high doses, should be avoided in the acute phase of injury. In the chronic phase, however, even a moderate dose of morphine leads to increased morphine intake in injured animals, suggesting that a state of neuropathic pain does not reduce the addictive potential of morphine. Thus, the use of opioids analgesics should be closely monitored in this phase of injury. As one of few effective analgesics available for the treatment of pain after SCI, it is difficult to suggest that morphine be removed as a potential treatment for the injured population. Instead, future studies must focus on the molecular mechanisms underlying morphine administration, as well as the potential synergistic actions of opioids and SCI pathology to negatively affect addiction, locomotor recovery, pain, and general health.⁷⁰

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Author Disclosure Statement

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Address correspondence to:

Sarah A. Woller, PhD

Department of Neuroscience and Experimental Therapeutics

Texas A&M University

MS 1359

Bryan, TX 77807-3260

E-mail: swoller@ucsd.edu