

# Opioid Facilitation of Rewarding Electrical Brain Stimulation Is Suppressed in Rats with Neuropathic Pain

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## ABSTRACT

**Introduction:** Opioids are powerful analgesics, but are also common drugs of abuse. Few studies have examined how neuropathic pain alters the pharmacology of opioids in modulating limbic pathways that underlie abuse liability.

**Methods:** Rats with or without spinal nerve ligation (SNL) were implanted with electrodes into the left ventral tegmental area and trained to lever press for electrical stimulation. The effects of morphine, heroin, and cocaine on facilitating electrical stimulation of the ventral tegmental area and mechanical allodynia were assessed in SNL and control subjects.

**Results:** Responding for electrical stimulation of the ventral tegmental area was similar in control and SNL rats. The frequency at which rats emitted 50% of maximal responding was  $98.2 \pm 5.1$  (mean  $\pm$  SEM) and  $93.7 \pm 2.8$  Hz in control and SNL rats, respectively. Morphine reduced the frequency at which rats emitted 50% of maximal responding in control (maximal shift of  $14.8 \pm 3.1$  Hz), but not SNL ( $2.3 \pm 2.2$  Hz) rats. Heroin was less potent in SNL rats, whereas cocaine produced similar shifts in control ( $42.3 \pm 2.0$  Hz) and SNL ( $37.5 \pm 4.2$  Hz) rats.

**Conclusions:** Nerve injury suppressed potentiation of electrical stimulation of the ventral tegmental area by opioids,

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## What We Already Know about This Topic

- Use of opioids to treat patients with chronic pain can be associated with addiction
- Animal models have been developed to study chronic pain and drug addiction

## What This Article Tells Us That Is New

- In normal animals, opioids facilitate the brain's reward system, whereas this effect is less in rats with chronic neuropathic pain
- In animals, the reinforcing effects of opioids are reduced in a chronic pain state

suggesting that the positive reinforcing effects are diminished by chronic pain. Given concerns regarding prescription opioid abuse, developing strategies that assess both analgesia and abuse liability within the context of chronic pain may aid in determining which opioids are most suitable for treating chronic pain when abuse is a concern.

TREATMENT of neuropathic pain with opioids remains controversial because of concerns regarding abuse potential.<sup>1</sup> These concerns are highlighted by the fact that treatment of neuropathic pain typically requires much larger doses of opioids than those used to treat acute pain.<sup>2</sup> Although much effort has been spent developing animal models to study the pathophysiology of this disease,<sup>3</sup> few studies have addressed the extent to which neuropathic pain alters the rewarding effects of opioids, which likely underlie opioid misuse in this population. This study addressed this issue by assessing opioid facilitation of rewarding electrical brain stimulation in rats with and without neuropathic pain.

Neuropathic pain suppresses the reinforcing effects of opioids in rodents. Dose-response curves for  $\mu$ -opioid receptor (MOR) agonists in maintaining self-administration are shifted to the right in nerve-injured rats, and only doses that

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alleviate mechanical allodynia maintain self-administration after nerve injury.<sup>4</sup> Similarly, nerve injury decreases morphine's ability to induce conditioned place preference, a paradigm thought to be an indirect measure of a drug's rewarding effects, in both rats<sup>5</sup> and mice.<sup>6</sup> A substantial body of literature implicates dopamine in mediating the rewarding effects of many abused drugs, including opioids. Specifically, dopaminergic neurons of the ventral tegmental area (VTA) project extensively to the nucleus accumbens, and release of dopamine in this region is thought to represent a key neural substrate for reward.<sup>7</sup> The VTA contains MORs located predominantly on nondopaminergic cells<sup>8</sup> and opioids increase the firing of VTA dopaminergic neurons,<sup>9</sup> presumably *via* disinhibition of dopaminergic cell bodies within the VTA. Previous work reveals that dopamine release in the nucleus accumbens after systemic administration of morphine is suppressed in nerve-injured rats.<sup>5</sup> Collectively, these findings suggest that neuropathic pain alters classic reward circuitry in rodents, resulting in altered opioid pharmacology.

One particularly useful method to study opioid activity within the VTA is intracranial electrical self-stimulation (ICSS). ICSS is an operant paradigm that pairs an operant response with brief electrical stimulation of a discrete brain region. Rats will lever press to receive electrical stimulation of the VTA, which causes a substantial release of dopamine in the nucleus accumbens.<sup>10</sup> Under these conditions, responding is directly related to the intensity and frequency of stimulation, such that intensity- and frequency-response curves can be generated that mirror pharmacological dose-response curves.<sup>11,12</sup> Pharmacology studies reveal that dopamine and MOR agonists facilitate VTA ICSS, indicated by their ability to produce leftward shifts in VTA ICSS frequency-response curves in rats.<sup>13</sup> To this end, ICSS has proven to be a valuable tool for assessing the effects of drugs or environmental conditions on a discrete pathway within the limbic system, which is difficult to do with systemic drug self-administration. Therefore, the application of this technique to a physiologic or pharmacological question can complement drug self-administration studies.

The effect of spinal nerve ligation (SNL) on rewarding electrical brain stimulation and modulation by opioids has not been documented. Given that previous work suggests that morphine is less effective in stimulating dopamine activity within the limbic system after nerve injury,<sup>5</sup> we hypothesized that opioid facilitation of VTA ICSS would be suppressed in nerve-injured rats. We therefore assessed the ability of the MOR agonists, morphine and heroin, to shift VTA ICSS frequency-response curves to the left in rats with and without neuropathic pain. In addition, the antiallodynic effects of each drug were assessed using von Frey filaments. In addition, facilitation of VTA ICSS by cocaine was examined in SNL and control rats to determine whether the effects of neuropathic pain were selective for opioids or would produce a generalized effect on this system.

## Materials and Methods

### Subjects

Subjects consisted of 26 male Fisher 344 rats (9 SNL rats and 11 control rats) were used exclusively for VTA ICSS, 4 SNL rats were used exclusively for determining the effects of drugs on paw withdrawal threshold [PWT], and 2 SNL rats were used for both VTA ICSS and determining drug effects on PWT). Rats weighed 300–350 g at the start of the experiment (Harlan Laboratories, Raleigh, NC), were group-housed, and were maintained on a reversed light-dark cycle (dark 5:00 AM to 5:00 PM) in a temperature- and humidity-controlled environment immediately adjacent to the room in which all behavioral experiments were performed. Food and water were available *ad libitum*, except during behavioral testing. All procedures were conducted in accord with the guidelines adopted by the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Animal Care and Use Committee of Wake Forest University School of Medicine (Winston-Salem, North Carolina).

### Surgeries

**Electrode Implantation.** Rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneal) and atropine methyl nitrate (10 mg/kg, intraperitoneal). Rats were placed in a stereotaxic frame, and platinum bipolar stimulating electrodes (Plastics One, Roanoke, VA) were implanted into the left VTA at a 10-degree angle (2.3 mm anterior to  $\lambda$ , 0.6 mm lateral from the midline, and 8.5 mm below the skull surface). Electrodes were permanently secured to the skull by three stainless-steel screws embedded in dental acrylic. Rats were given penicillin G procaine (75,000 U, intramuscular) to prevent postsurgical infection.

**Spinal Nerve Ligation.** Immediately after electrode implantation, a portion of rats were subjected to SNL.<sup>14</sup> In brief, a 3-cm incision was made in the back using the iliac crests as a midpoint. An incision was then made in the underlying muscle, which was separated by both sharp and blunt dissection to expose the left transverse process of the fifth lumbar vertebra. The transverse process was removed using bone micro-rongeurs, and the fifth lumbar nerve was exteriorized from underneath the spinal column using a small metal hook and ligated using a 4.0 silk suture with sufficient pressure to cause the nerve to bulge on each side of the ligature. The sixth lumbar nerve was exteriorized from underneath the iliac bone at the sciatic notch and ligated in a similar manner. All muscle layers were sutured using 4.0 chromic gut, the skin was sutured using a 4.0 nylon suture, and exterior wounds were dressed with antibiotic powder (Polysporin; Pfizer Healthcare, Morris Plains, NJ).

### Paw Withdrawal Threshold

To verify development of mechanical allodynia following SNL, PWTs were determined according to previously pub-

lished methods using von Frey filaments ranging in strength from 0.6 to 26.0 g (Touch Test Sensory Evaluators; Stoelting, Wood Dale, IL)<sup>15</sup> for all animals using Dixon nonparametric statistics.<sup>16</sup> After a minimum of 14 days recovery from electrode implantation and SNL, withdrawal thresholds were determined and rats were considered allodynic if the withdrawal threshold was 4.0 g or less; development of allodynia was a requisite for inclusion of SNL rats in the current study. To determine whether the drugs used during VTA ICSS alleviated established mechanical allodynia, baseline PWTs were determined 20 min before intraperitoneal drug injections. PWTs were then assessed 15, 60, and 120 min postinjection in a portion of rats subjected to SNL.

### Drugs

Morphine sulfate was purchased as a 15-mg/ml sterile solution (Baxter Healthcare, Deerfield, IL). Heroin hydrochloride and cocaine hydrochloride were obtained from the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD), dissolved in 0.9% (wt/vol) saline, and sterilized by filtration through a 0.22- $\mu$ m nitrocellulose filter. All drugs were diluted using 0.9% (wt/vol) saline (pH 7.4).

### ICSS

**Apparatus.** Commercially available operant equipment was used, consisting of an operant chamber containing a lever located 5 cm above a grid-bar floor, a stimulus lamp located 2 cm above the lever, a house light located outside of the operant chamber, and a tone generator (Med Associates, Inc., St. Albans, VT). The operant chamber was housed within a sound- and light-attenuating enclosure containing a ventilation fan. An ICSS stimulator controlled by a computer software program version 4 (Med Associates) that controlled all stimulation parameters and data collection was located outside of the enclosure. A two-channel swivel commutator (model SLC2C; Plastics One) located above the operant chamber connected the electrodes to the ICSS stimulator *via* 25-cm cables (Plastics One).

**Behavioral Procedure.** After a minimum of 14 days of recovery from surgery, rats were trained to lever press for brain stimulation. Illumination of the stimulus light above the lever indicated stimulation availability. Each lever press resulted in a 0.5-s train of rectangular alternating cathodal and anodal pulses (0.1-ms pulse durations) and was accompanied by the stimulus light turning off, the houselight illuminating, and operation of the tone. Responses during the 0.5-s stimulation period provided no additional stimulation and were not recorded.

To determine the lowest stimulation intensity (current) that maintained high rates of responding, daily intensity-rate curves were generated. These 1-h sessions consisted of six 10-min components, with each component subdivided into ten 1-min trials. Each trial began with a 5-s timeout period, a 5-s priming period in which rats received five noncontingent stimulations, and a 50-s response period in which lever presses resulted in stimulation and were recorded. During

these sessions, the frequency of stimulation was held constant (156 Hz), and a series of 10 intensities (200–20  $\mu$ A, 20- $\mu$ A increments corresponding to each trial) were presented in descending order. For data analysis, response rates during the first two components of each session were discarded, because they were often highly variable and response rates during components 3–6 were averaged to create a single intensity-rate curve for each session. For each session, the intensity that maintained 80% of maximal responding ( $EC_{80}$ ) was determined using Prism software version 4 (sigmoidal-dose response, variable slope; GraphPad, Inc., La Jolla, CA), and responding was deemed stable when the  $EC_{80}$  values of three consecutive sessions varied by less than 10% of the average  $EC_{80}$  value of the three sessions. The average  $EC_{80}$  value (intensity) of these three sessions was used for frequency-rate curve sessions and was adjusted, if needed.

Daily frequency-rate curves were generated similarly to the intensity-rate curves, except that the intensity was held constant (unique to each animal) and a series of 10 frequencies (156–45 Hz, 0.06 log increments corresponding to each trial) were presented in descending order. Test sessions consisted of seven components with a 15- or 60-min time-out period between components 4 and 5, during which time rats received 1-ml/kg intraperitoneal injections of saline (0.9% wt/vol), morphine (0.3–6 mg/kg), heroin (0.03–1 mg/kg), or cocaine (0.3–10 mg/kg). For data analysis, the two components preceding drug injection (components 3 and 4) and the three components following drug injection (components 5, 6, and 7) were averaged and compared using Prism software (sigmoidal-dose response, variable slope; GraphPad). Drug-test sessions were separated by at least 1 day. Saline was administered to each animal first, followed by administration of morphine or cocaine on alternate testing days. Heroin was tested after the morphine and cocaine dose-effect curves were completed, followed finally by testing effective doses of morphine at the 60-min pretreatment time. Preliminary experiments were performed to determine the highest possible doses of each drug that could be administered without decreasing maximum response rates for VTA ICSS (data not shown in Results). All test sessions were performed between 1 and 5 months postsurgery in both groups of rats. Additional animals were added to the study, as needed, due to attrition from electrode loss or decreases in baseline responding for VTA ICSS with time.

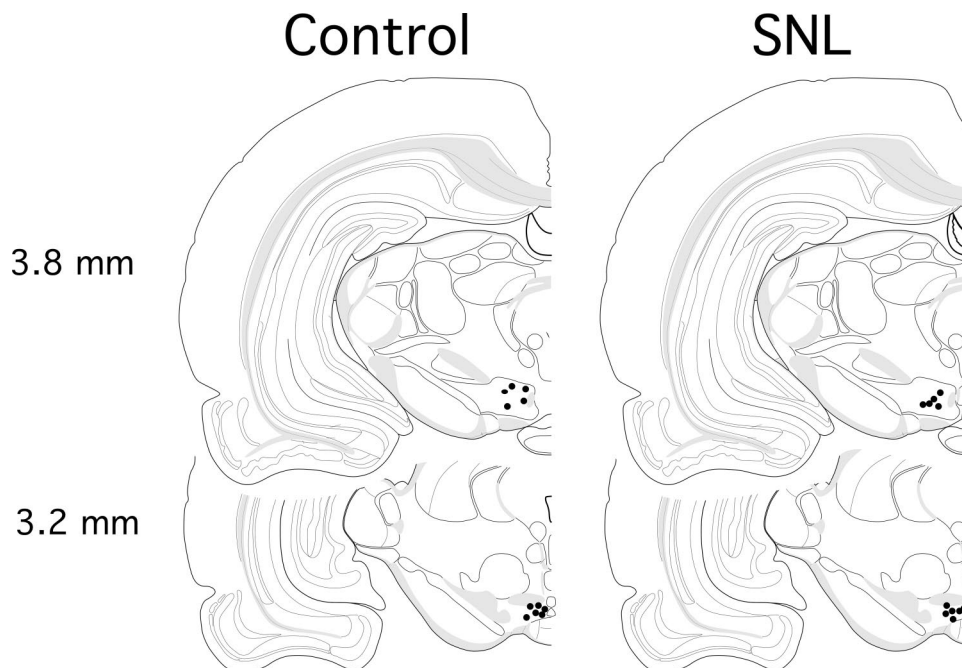
### Histology

Rats were sacrificed by carbon-dioxide asphyxiation. Brains were rapidly removed and frozen in isopentane ( $-35^{\circ}\text{C}$ ) and were stored at  $-80^{\circ}\text{C}$ . Coronal sections (25  $\mu$ m) around the electrode tract were obtained using a cryostat to confirm electrode placement within the VTA (fig. 1).

### Statistical Analysis

Data for PWTs were analyzed using a two-way ANOVA, with drug dose and time after infusion serving as the inde-





**Fig. 1.** Schematic showing the location of stimulating electrodes within the ventral tegmental area for control and spinal nerve-ligated (SNL) rats. Numbers next to diagrams indicate the distance anterior to the interaural line according to the atlas of Paxinos and Watson.<sup>24</sup>

pendent variables. The  $EF_{50}$  value (frequency at which rats emitted 50% of maximal responding) and maximum response rate for VTA ICSS were calculated using Prism software (sigmoidal-dose response, variable slope; GraphPad, Inc.). The effect of drug treatment and SNL on VTA ICSS was analyzed using a two-way ANOVA, with drug dose and treatment condition (SNL or control) serving as the independent variables and  $\Delta EF_{50}$  ( $EF_{50}$  value before injection –  $EF_{50}$  value after injection) or maximal response rates serving as the dependent measures. *Post-hoc* analyses within the control or SNL groups were made using a Dunnett *t* test for multiple comparisons, with saline injection serving as control. *Post-hoc* comparisons between control and SNL groups were made using Tukey HSD. A two-tailed *P* value of 0.05 or less was considered statistically significant.

## Results

### Development of Mechanical Allodynia

PWTs were significantly different between control and SNL rats implanted with VTA electrodes [ $F(1,21) = 71.0$ ,  $P < 0.0001$ ]. All rats implanted with VTA electrodes and subjected to SNL developed mechanical allodynia, with average PWTs of  $2.21 \pm 0.25$  g (mean  $\pm$  SEM; fig. 2). Rats implanted with VTA electrodes, but not subjected to SNL, did not develop mechanical allodynia, with average PWTs of  $15.15 \pm 1.51$  (fig. 2).

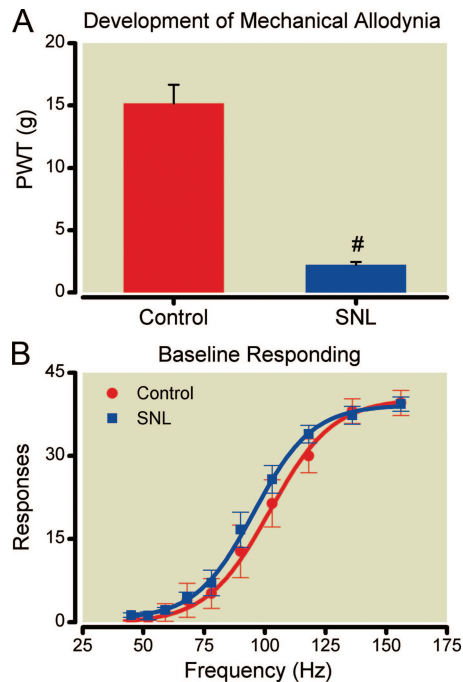
### Intracranial Self-stimulation

**Effect of SNL on Baseline VTA ICSS.** Electrical stimulation maintained responding in a frequency-dependent manner in

both control and SNL rats. There was no significant effect of SNL on VTA ICSS, compared to control rats, with either the  $EF_{50}$  or the maximal response rate (fig. 2). The  $EF_{50}$  for VTA ICSS in SNL rats was  $93.7 \pm 2.8$  Hz and for control rats was  $98.3 \pm 5.1$  Hz [ $F(1,20) = 0.6$ ,  $P = 0.43$ ]. The maximal response rate in SNL rats was  $38.4 \pm 1.4$  resp/trial and for control rats was  $38.3 \pm 2.3$  resp/trial [ $F(1,20) = 0.001$ ,  $P = 0.98$ ].

**Morphine.** The effect of 15-min pretreatment with morphine in shifting the frequency-response curves to the left was significantly different between control and SNL rats [ $F(1,80) = 6.1$ ,  $P = 0.02$ ], and this effect was dose dependent [ $F(4,80) = 5.1$ ,  $P = 0.001$ ] (fig. 3). There was a significant interaction between SNL *versus* control and morphine dose [ $F(4,80) = 2.7$ ,  $P = 0.03$ ]. In the control group, morphine significantly increased  $\Delta EF_{50}$  values at all doses of 1 mg/kg and greater, compared to saline [ $F(4,41) = 5.3$ ,  $P = 0.002$ ]. In contrast, in the SNL group, morphine did not significantly alter the  $\Delta EF_{50}$  value, compared to saline at any dose [ $F(4,38) = 0.8$ ,  $P = 0.54$ ], and saline alone did not alter the  $\Delta EF_{50}$  values in either group ( $P > 0.05$ ).

Morphine's effect on ICSS in the VTA in control rats was greater after 1 h, compared to 15 min pretreatment; however, morphine was still without effect in SNL rats (fig. 3). Analysis of the data with the longer morphine pretreatment time revealed a similar effect [ $F(9,77) = 6.4$ ,  $P < 0.0001$ ], with morphine's effect being dose responsive [ $F(4,77) = 7.3$ ,  $P < 0.0001$ ] and SNL producing a significant effect on the ability of morphine to shift the  $EF_{50}$  [ $F(1,77) = 9.4$ ,  $P = 0.003$ ]. As with the earlier time point, there was a significant interaction



**Fig. 2.** Development of mechanical allodynia after spinal nerve ligation (SNL) and baseline responding for electrical stimulation of the ventral tegmental area in control and SNL rats. (A) Paw withdrawal thresholds (PWTs) were assessed between 14–21 days after surgery using von Frey filaments in control and SNL rats. (B) Frequency-response curves for baseline responding were generated by averaging the third and fourth components preceding saline administration (before any drug treatments). The y-axis indicates the number of self-stimulations (0.5-s) during each 50-s trial for each frequency (x-axis). Data shown are averages across control ( $n = 11$ ) and SNL ( $n = 11$ ) rats. Average current intensities during baseline responding were 139.6  $\mu$ A (14.23) for control and 138.6  $\mu$ A (9.83) for SNL rats. #Significantly different from control rats ( $P \leq 0.05$ ).

between SNL *versus* control and morphine dose [ $F(4,77) = 4.3$ ,  $P = 0.004$ ]. In control rats, morphine produced significant leftward shifts in the frequency-response curves, and  $\Delta EF_{50}$  values were dose dependent [ $F(4,40) = 8.9$ ,  $P < 0.001$ ], with all doses of 1 mg/kg and greater producing a significant effect. As with the earlier pretreatment time, morphine had no effect on the  $\Delta EF_{50}$  values at any dose in SNL rats [ $F(4,36) = 1.0$ ,  $P = 0.4$ ], and saline alone did not alter the  $\Delta EF_{50}$  values in either group following a 1-h pretreatment ( $P > 0.05$ ).

**Heroin.** Fifteen-minute pretreatment with heroin altered ICSS in the VTA in both control and SNL rats; however, significant differences were found between these two groups (fig. 4). Heroin shifted the frequency-response curves to the left, producing significant increases in the  $\Delta EF_{50}$  values in a dose-responsive manner in both control [ $F(4,35) = 6.5$ ,  $P = 0.0006$ ] and SNL [ $F(4,41) = 8.1$ ,  $P < 0.0001$ ] rats. In the control group, heroin significantly increased  $\Delta EF_{50}$  values at all doses of 0.1 mg/kg and greater, compared to saline ( $P \leq 0.05$ ). In the SNL group, heroin significantly increased

$\Delta EF_{50}$  values at all doses of 0.3 mg/kg and greater, compared to saline ( $P \leq 0.05$ ).

**Cocaine.** Fifteen-minute pretreatment with cocaine altered ICSS in the VTA in both control and SNL rats in a similar manner (fig. 5). Cocaine shifted the frequency-response curves to the left, producing significant increases in the  $\Delta EF_{50}$  values in a dose-responsive manner in both groups of rats [control:  $F(4,36) = 42.5$ ,  $P < 0.0001$ ; SNL:  $F(4,41) = 40.1$ ,  $P \leq 0.0001$ ]. Cocaine significantly increased  $\Delta EF_{50}$  values at all doses of 1 mg/kg and greater, compared to saline ( $P \leq 0.05$ ). There were no significant differences between control and SNL rats for any dose of cocaine.

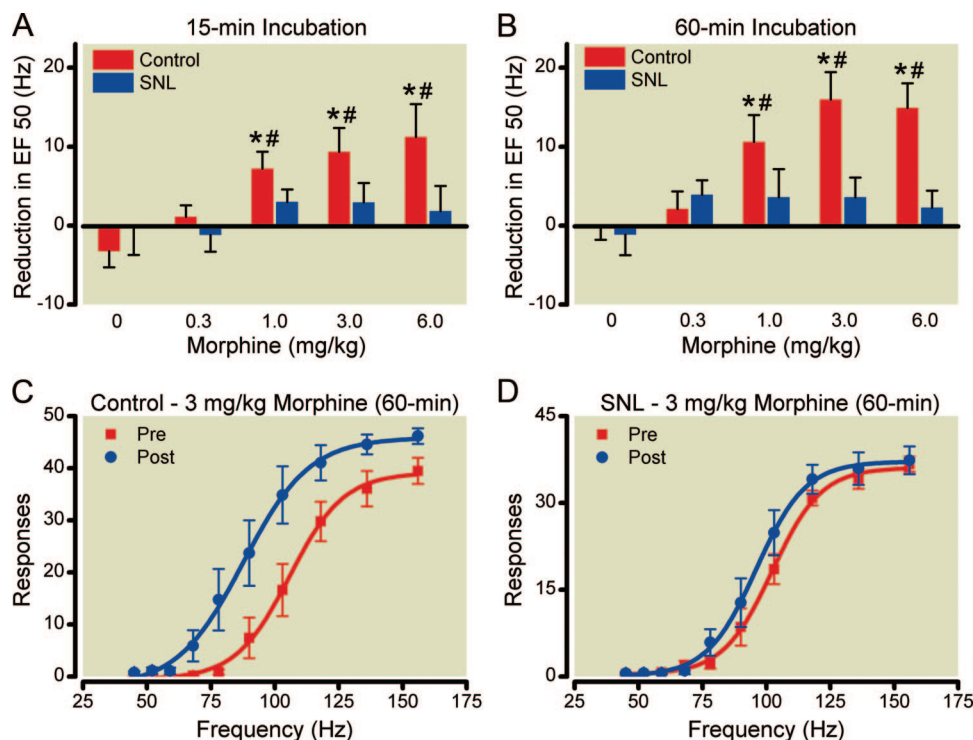
**Drug Effects on Maximal Response Rate.** Both morphine and heroin had no effect on the maximum rate of responding maintained by intracranial stimulation in both groups at any time following injection. Cocaine had no effect on the maximum rate of responding in the control group [ $F(4,36) = 1.0$ ,  $P = 0.4$ ], but did slightly increase the maximum response rate in the SNL group [ $F(4,41) = 2.8$ ,  $P = 0.04$ ] at the highest dose studied of 10 mg/kg ( $39.5 \pm 1.4$  baseline,  $43.5 \pm 2$  treated,  $P < 0.05$ ).

### Drug Effects on Mechanical Allodynia

Morphine and heroin both increased PWT over the range of doses that produced significant increases in  $\Delta EF_{50}$  values for ICSS (fig. 6). The effects of morphine were dose dependent [ $F(3,23) = 15.6$ ,  $P < 0.0001$ ;  $F(3,23) = 27.2$ ,  $P < 0.0001$ , 15 or 60 min postinjection, respectively]. Both 3 and 6 mg/kg of morphine significantly increased PWT at these time points, compared to saline injection ( $P < 0.05$ ), and the maximum effect occurred 60 min after injection of 6 mg/kg of morphine, resulting in a PWT of  $16.6 \pm 2.1$  g. Heroin also produced dose-dependent increases in PWT [ $F(3,23) = 38.4$ ,  $P < 0.0001$ ;  $F(3,23) = 22.8$ ,  $P < 0.0001$ , 15 or 60 min postinjection, respectively]. Both 0.3 and 1.0 mg/kg of heroin significantly increased PWT at these time points, compared to saline ( $P < 0.05$ ), and the maximum effect of heroin ( $22.1 \pm 0.8$  g) was greater than that of morphine, occurring 15 min after injection of 1 mg/kg ( $P \leq 0.05$ ). Cocaine had no effect on PWT at any dose [ $F(2,17) = 1.6$ ,  $P = 0.2$ ;  $F(2,17) = 1.1$ ,  $P = 0.4$ , 15 or 60 min postinjection, respectively] (fig. 6). Saline had no effect on PWT at any time after injection [ $F(2,17) = 0.2$ ,  $P = 0.8$ ] (fig. 6).

### Discussion

Given the widespread concerns regarding opioid misuse in chronic pain patients, there is a need to determine to what extent the presence of chronic pain alters the abuse potential of opioids. The current study indicates that MOR agonists become less effective in facilitating VTA ICSS after peripheral nerve injury, suggesting that their ability to produce positive reinforcement is diminished by the presence of chronic pain. The diminished effects in SNL rats appear to be specific to opioids, because cocaine was equally effective in facilitating VTA ICSS in both control and SNL rats. These



**Fig. 3.** Effects of spinal nerve ligation (SNL) on morphine facilitation of electrical stimulation of the ventral tegmental area for drug-incubation times of 15 (A) and 60 min (B). Reduction in  $EF_{50}$  (frequency at which rats emitted 50% of maximal responding) was calculated by subtracting the  $EF_{50}$  values for the three components following drug injection (components 5–7) from the  $EF_{50}$  value for the two components preceding drug injection (components 3–4) for each dose assessed. Data shown are averages across control ( $n = 7$ –8) and SNL ( $n = 6$ –7) rats. Frequency-response curves before and after 3 mg/kg of morphine (60-min) are shown for control (C;  $n = 8$ ) and SNL (D;  $n = 7$ ) rats. \*Significantly different from saline treatment  $P \leq 0.05$ ; #significantly different from SNL rats ( $P \leq 0.05$ ).

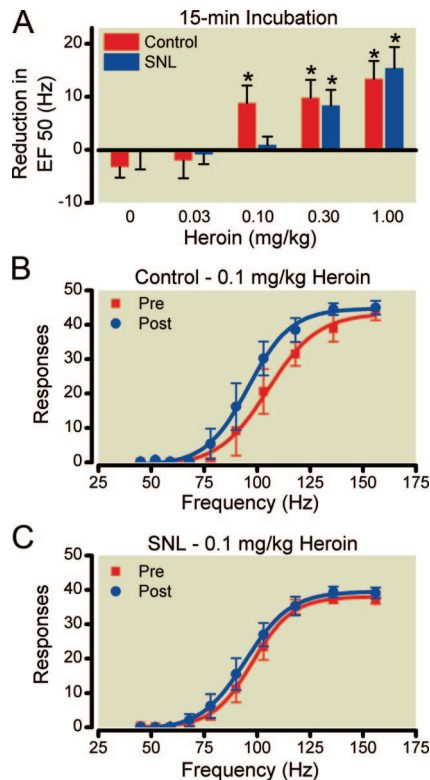
data support our hypothesis that the efficacy of opioids in stimulating the mesolimbic dopaminergic system is suppressed in rats with neuropathic pain.

It is important to highlight differences between the effects of SNL on suppressing morphine and heroin facilitation of ICSS. The facilitating effects of morphine were suppressed to such an extent following SNL that no dose of morphine (0.3–6 mg/kg) produced leftward shifts in the frequency-response curves after SNL. The complete suppression of morphine's facilitating effects in SNL rats after 15-min drug incubation was surprising; to further confirm these findings, a 60-min pretreatment was used, which produced even greater leftward shifts in the frequency-rate curves in control subjects, and, again, morphine failed to facilitate ICSS selectively in SNL rats. In contrast, only one dose of heroin assessed (0.1 mg/kg) produced a significant leftward shift in control rats that was not observed in SNL rats; meanwhile, heroin was still effective in facilitating VTA ICSS at the two highest doses assessed (0.3 and 1 mg/kg) in control and SNL rats. Doses higher than 6 mg/kg of morphine and 1 mg/kg of heroin decreased maximal response rates for ICSS (data not shown), and therefore the dose range was restricted to 6 and 1 mg/kg, respectively. From these data, one would predict that morphine and heroin would produce positive reinforcement in control rats, but that only heroin would produce

positive reinforcement in SNL rats, albeit at larger doses. Interestingly, this prediction is supported by previous work that assessed opioid self-administration in rats with and without SNL.<sup>4</sup> In those experiments, SNL decreased maximal response rates for morphine self-administration, compared to SHAM rats, to such an extent that responding for morphine in SNL rats no longer was dose dependent; morphine essentially appeared to not serve as a reinforcer after SNL. In contrast, heroin self-administration was merely shifted to the right in SNL, compared to SHAM, rats, and maximal rates of responding were unaffected; heroin still served as a reinforcer at higher doses following SNL. Taken together, suppression of the reinforcing effects of opioids in each of these paradigms seems to be related to the efficacy of the opioid, such that the reinforcing effects of lower efficacy opioids (*i.e.*, morphine) are completely diminished after SNL, whereas those of higher efficacy opioids (*i.e.*, heroin) can be overcome by increasing the dose.

The mechanism underlying the loss of opioid facilitation of VTA ICSS following SNL appears to not be general disruption of limbic activity. The ability of electrical stimulation of the VTA to maintain operant responding is not altered by SNL; the frequency-response curves and the mean intensities required to maintain responding did not differ between SNL and control rats. In addition, the facilitating

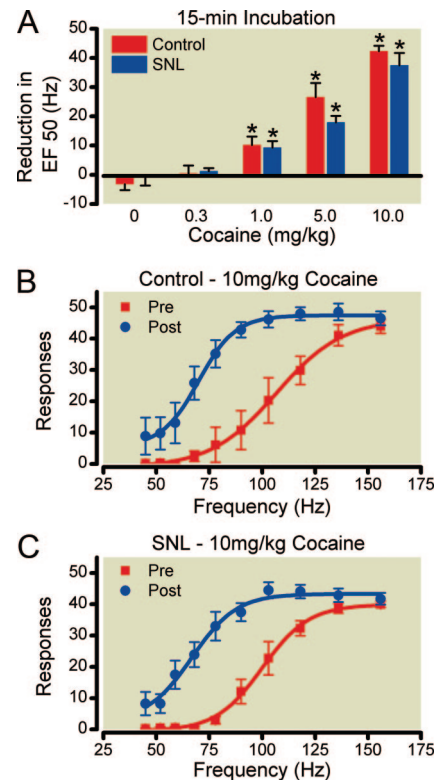




**Fig. 4.** Effects of spinal nerve ligation (SNL) on heroin facilitation of electrical stimulation of the ventral tegmental area. (A) Reduction in EF<sub>50</sub> (frequency at which rats emitted 50% of maximal responding) was calculated by subtracting the EF<sub>50</sub> value for the three components following drug injection (components 5–7) from the EF<sub>50</sub> value for the two components preceding drug injection (components 3–4) for each dose assessed. Data shown are averages across control (*n* = 6–7) and SNL (*n* = 7–8) rats. Frequency-response curves before and after 0.1 mg/kg heroin (15-min) are shown for control (B; *n* = 6) and SNL (C; *n* = 8) rats. \*Significantly different from saline treatment (*P* ≤ 0.05).

effects of cocaine on VTA ICSS were the same in SNL and control rats, suggesting that SNL does not cause a nonspecific disruption in behavior or limbic activity. Therefore, the effects appear to be unique to opioids, leaving the possibility that SNL produces a selective disruption in MOR activity. In particular, alterations in MOR G-protein signaling may occur in the VTA, leading to reduced signaling after MOR-agonist activation and, ultimately, suppression of opioid facilitation of VTA ICSS. Previous work supports this notion, because it was shown that morphine-stimulated GTPγS binding in the VTA and morphine-induced dopamine release in the nucleus accumbens are suppressed in nerve-injured rats.<sup>5</sup> Similar effects were observed in nerve-injured mice<sup>17</sup>; however, the mechanism for receptor uncoupling within this circuitry is unclear. It is also unclear whether such uncoupling could be reversed with acute or chronic blockade of pain transmission with analgesics, or if the alterations are irreversible.

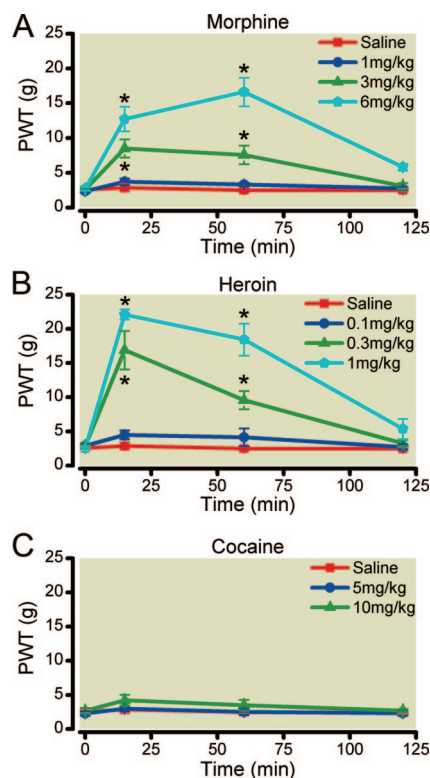
MOR signaling may be altered after SNL in other brain regions that influence activity within the VTA or receive



**Fig. 5.** Effects of spinal nerve ligation (SNL) on cocaine facilitation of electrical stimulation of the ventral tegmental area. (A) Reduction in EF<sub>50</sub> value (frequency at which rats emitted 50% of maximal responding) was calculated by subtracting the EF<sub>50</sub> value for the three components following drug injection (components 5–7) from the EF<sub>50</sub> value for the two components preceding drug injection (components 3–4) for each dose assessed. Data shown are averages across control (*n* = 6–7) and SNL (*n* = 7–8) rats. Frequency-response curves before and after 10 mg/kg heroin (15-min) are shown for control (B; *n* = 7) and SNL (C; *n* = 8) rats. \*Significantly different from saline treatment (*P* ≤ 0.05).

output from the VTA. Other brain regions that would most likely be involved are those receiving dopaminergic output from the VTA, including the nucleus accumbens, ventral pallidum, and amygdala,<sup>7</sup> all of which contain MORs. To address this, future studies could examine the degree to which chronic pain alters the facilitating effects of opioids administered locally into each of these discrete brain regions. Given the knowledge of which MORs are and are not responsible for this effect, the underlying neural mechanisms responsible for loss of opioid efficacy in facilitating VTA ICSS could be explored.

The suppression of opioid facilitation of rewarding brain stimulation after nerve injury has important implications regarding opioid abuse potential in the presence of chronic pain. Most drugs of abuse, including opioids, increase dopamine transmission throughout the mesolimbic dopaminergic system,<sup>13</sup> and their ability to facilitate VTA ICSS is often used as a measure of their abuse potential. Therefore, a reduction in the facilitating effects of opioids on VTA ICSS



**Fig. 6.** Drug effects on paw withdrawal thresholds (PWTs) in spinal nerve-ligated (SNL) rats. Dose-effect curves were determined for morphine (A), heroin (B), and cocaine (C) on PWT in SNL rats at the indicated times after an intraperitoneal injection ( $n = 6$ ). \*Significantly different from saline treatment ( $P \leq 0.05$ ).

after SNL indicates a loss of the positive rewarding effects that likely contribute to opioid abuse. Clinically, the notion that opioid abuse potential is reduced by the presence of chronic pain is not new. A recent review suggests that abuse/addiction rates in chronic-pain patients are relatively low ( $\sim 3\%$ ),<sup>18</sup> although precise rates for opioid misuse remain difficult to determine.

Because this is the first report of opioid effects on VTA ICSS during chronic pain, it is unclear whether similar suppression of VTA ICSS would be observed with other opioid compounds. In addition, it is unclear to what extent drug history alters the facilitatory effects of opioids on VTA ICSS. In the current study, rats received all drug conditions, and although repeated testing could potentially affect facilitation of ICSS, the fact that drug history was similar between control and SNL rats suggests that these effects are likely negligible. In addition, morphine injections were given at the beginning of the study at the 15-min time point and at the end of the study at the 60-min pretreatment condition. Similar potencies were found for facilitation of VTA ICSS in the control group, and morphine had no effect on VTA ICSS in the SNL group, suggesting that drug history likely had a minor role, if any, on these data. An interesting question with respect to drug history would be whether extensive drug exposure (*i.e.*, months) before nerve injury would alter the

abuse liability of opioids, as measured using VTA ICSS. This question is particularly interesting, given that one of the greatest predictors of opioid abuse/addiction in chronic-pain patients is a previous history of drug abuse.<sup>18</sup> Ultimately, VTA ICSS has great potential as a preclinical screening tool for assessing the abuse liability of potential opioid as well as nonopioid analgesics during chronic pain.

It was surprising that no differences were observed in baseline responding for VTA ICSS between control and SNL rats. Administration of lactic acid into the peritoneal cavity produces abdominal pain and irritation and has recently been shown to suppress responding for ICSS, an effect that is reversed by pretreatment with morphine.<sup>19</sup> These data suggest that the presence of severe acute pain diminishes the reinforcing effects of electrical stimulation of ascending dopamine pathways. Lactic acid injected into the peritoneal cavity produces overt abdominal writhing and stretching behavior, and rats will typically cease ongoing behavior during these episodes, which are inhibited by a variety of analgesics, including opioids.<sup>19</sup> SNL produces few overt behavioral effects, however. The difference between the effects of intraperitoneal lactic acid and SNL on ICSS may be the presence of these pronounced behavioral effects, the relative severity of the pain stimulus, or differential effects on the mesolimbic reward pathways. Given that people with chronic pain often suffer from affective disorders, such as anxiety and depression,<sup>20</sup> which suggests that persistent pain leads to an overall negative affective state, it was expected that nerve injury would reduce activity of reward pathways originating within the limbic system, and therefore that SNL rats would be less responsive to rewarding electrical stimulation. However, the current data suggest that if SNL decreases activity within the limbic system in rats, VTA ICSS lacks the sensitivity to detect such an effect of this manipulation. Given that the central amygdala is activated during pain states<sup>21,22</sup> and sends GABAergic projections that synapse directly on dopaminergic cell bodies of the VTA,<sup>23</sup> it is plausible that chronic pain increases the activity of these central amygdala GABAergic neurons, causing tonic suppression of dopamine transmission from the VTA in neuropathic rats. The present data suggest, however, that the basal activity of reward pathways is not significantly inhibited after SNL, at least not to an extent that can be detected using the ICSS methodology. Instead, the present data suggest that dopaminergic reward pathways originating from the VTA are altered in a highly selective manner after SNL in rats, resulting in a specific alteration in opioid pharmacology within this circuitry.

In conclusion, the ability of opioids to facilitate VTA ICSS is suppressed following SNL. This suggests that opioids are less effective in stimulating dopamine transmission originating in the VTA. The effect appears to be restricted to opioids, because facilitation of VTA ICSS with cocaine is maintained in neuropathic rats. The current methodology used (VTA ICSS) not only complements previous work using drug self-administration, but adds an additional layer of



precision by isolating opioid effects within limbic reward circuitry, which is difficult to do using systemic drug self-administration. Future work should focus on whether similar effects occur with different opioid compounds, as well as determine the underlying mechanisms responsible for opioid suppression after nerve injury. The use of operant techniques, such as VTA ICSS, may be beneficial as a preclinical tool for screening the abuse liability of novel analgesics, as well as provide useful information regarding the interactions between chronic pain and classic reward circuitry.

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