ABSTRACT

Background: Prescription opioid abuse is a significant concern in treating chronic pain, yet few studies examine how neuropathic pain alters the abuse liability of commonly abused prescription opioids.

Methods: Normal and spinal nerve ligated (SNL) rats were implanted with electrodes into the left ventral tegmental area (VTA). Rats were trained to lever press for intracranial electrical stimulation (VTA ICSS), and the effects of methadone, fentanyl, hydromorphone, and oxycodone on facilitation of VTA ICSS were assessed. A second group of neuropathic rats were implanted with intrathecal catheters, and the effects of intrathecal clonidine, adenosine, and gabapentin on facilitation of VTA ICSS were assessed. The effects of electrical stimulation of the VTA on mechanical allodynia were assessed in SNL rats.

Results: Responding for VTA ICSS was similar in control and SNL rats. Methadone, fentanyl, and hydromorphone were less potent in facilitating VTA ICSS in SNL rats. Oxycodone produced a significant facilitation of VTA ICSS in control (maximum shift 24.10 ± 6.19 Hz) but not SNL rats (maximum shift 16.32 ± 7.49 Hz), but also reduced maximal response rates in SNL rats. Intrathecal administration of clonidine, adenosine, and gabapentin failed to facilitate VTA ICSS in SNL rats, and electrical stimulation of the VTA did not alter mechanical allodynia following nerve injury.

Conclusions: The present data suggests that the positive reinforcing effects of commonly abused prescription opioids are diminished following nerve injury. In addition, alleviation of mechanical allodynia with nonopioid analgesics does not appear to stimulate limbic dopamine pathways originating from the VTA in SNL rats.

SIGNIFICANT issues regarding the treatment of chronic pain with opioids remain because of concerns over misuse and diversion of medications to illicit drug markets.1,2 Prescription opioid abuse is now the fastest-growing drug abuse problem in the United States,3 and presents a significant economic burden.4 A recent study of patients seeking treatment for dependence on prescription opioids reports that the most commonly abused narcotics include oxycodone (79%), hydrocodone (67%), methadone (40%), morphine (29%), hydromorphone (16%), and fentanyl (9%).5 Few preclinical studies exist, however, that examine the abuse potential of these drugs in the context of pain.

Laboratory investigations aimed at understanding the interaction between pain and addiction have begun to provide some evidence that chronic pain suppresses opioid reward.6 Spinal nerve ligated (SNL) rats require larger doses of opioids to maintain intravenous self-administration.7 Nerve injury also decreases morphine-induced conditioned place prefer-
ence (CPP) in rats and mice. Similarly, direct injection of the μ-opioid receptor agonist DAMGO into the ventral tegmental area (VTA), an area implicated in opioid reward, produces CPP that is suppressed in nerve-injured mice.

Behavioral paradigms, such as self-administration and CPP, have been extensively used for assessment of drug reward, yet in the context of chronic pain, assessment of reward may be complicated by a drug’s analgesic effects. For instance, self-administration of opioids in SNL rats occurs only at doses that also alleviate mechanical allodynia, and administration of the spinal analgesic clonidine reduces opioid intake selectively in nerve-injured rats. Moreover, spinal clonidine alone produces CPP and maintains intrathecal self-administration selectively in nerve-injured rats at doses that alleviate mechanical allodynia. One explanation of these data is that alleviation of mechanical allodynia serves as a direct reinforcing stimulus in rats following nerve injury. Another possible explanation, however, is that alleviation of mechanical allodynia indirectly stimulates classic reward pathways thought to be primarily responsible for the abuse potential of drugs.

Intracranial self-stimulation (ICSS) is a technique that has been used to evaluate the rewarding effects of drugs. Rats can be trained to lever press for electrical stimulation of the VTA, which is directly influenced by the intensity and frequency of stimulation. VTA stimulation increases dopamine neurotransmission in the nucleus accumbens. The VTA is implicated in opioid reward; opioids increase the firing of VTA dopaminergic neurons via disinhibition, and subsequently reduce the intensity and/or frequency of stimulation required to maintain responding for VTA ICSS in rats (facilitation). Recently, we reported that morphine and heroin facilitation of VTA ICSS is suppressed in SNL rats.

Given the growing prescription opioid abuse problem, one goal of the current work was to examine the effects of nerve injury on the rewarding effects of commonly abused prescription opioids using VTA ICSS. Given that spinal clonidine elicits rewarding effects in animals with neuropathic pain, a second goal of the current work was to determine if alleviation of mechanical allodynia with analgesics stimulates the mesolimbic dopamine system using VTA ICSS in SNL rats.

Materials and Methods

Subjects

Subjects consisted of 42 male, Fisher 344 rats: Eight SNL and nine control rats for assessment of opioids on VTA ICSS; seven SNL rats for assessment of opioids on paw withdrawal threshold (PWT); four SNL rats for assessment of spinal analgesics on VTA ICSS and PWT, four SNL rats for assessment of morphine on VTA ICSS, and three SNL rats for both spinal analgesics and morphine; and seven control rats for assessment of morphine on VTA ICSS. Rats weighed between 300–350 g at the beginning of the experiment. All rats (Harlan Laboratories, Raleigh, NC) were group-housed, except for those receiving intrathecal catheters, and were maintained on a reversed light-dark cycle (dark 5:00 AM to 5:00 PM). Rats were housed in a temperature- and humidity-controlled room that was adjacent to the room where behavioral experiments were performed. Food and water were available ad libitum with the only exception being during behavioral experiments. All procedures were conducted according to guidelines of 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), and received penicillin G procaine (75,000 IU, intramascular) as a preventative measure against infection. After being placed in a stereotaxic frame, platinum bipolar stimulating electrodes (Plastics One, Roanoke, VA) were implanted into the left VTA at a 10° angle (2.3 mm anterior to lambda, 0.6 mm lateral from the midline, and 8.5 mm below the skull surface). Three stainless steel screws embedded in dental acrylic permanently secured each electrode to the skull surface.

Spinal Nerve Ligation. Immediately following electrode implantation, 26 of the 42 rats were subjected to SNL as previously described. A 3-cm incision was made through the skin and underlying muscle of the lower back, which was separated, and the left transverse process of the fifth lumbar vertebra was removed using bone microrongeurs. The fifth lumbar nerve was then exteriorized and ligated using 4.0 silk suture. The sixth lumbar nerve was exteriorized from below the iliac bone at the sacroiliac notch and similarly ligated. Each ligation caused the nerve to bulge on each side of the ligature. Muscle layers were sutured with 4.0 chromic gut, the skin with 4.0 nylon suture, and exterior wounds dressed with antibiotic powder (Polysporin; Pfizer Healthcare, Morris Plains, NJ).

Intrathecal Catheter Implantation. Rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneal) and atropine methyl nitrate (10 mg/kg, intraperitoneal), and received penicillin G procaine (75,000 IU, intramascular) as a preventive measure against infection. Rats were then implanted with intrathecal catheters according to previously described methods. Rats were briefly placed in a stereotaxic frame with the head bent downward. An incision was made through the skin and muscle of the neck, which was retracted, and a small hole was made in the atlanto-occipital membrane through which an 8.5-cm catheter was inserted. The spinal catheter was constructed from 32 g polyethylene tubing (ReCathCo, Allison Park, PA) fused to biocompatible Tygon tubing (ID 0.01 in., formulation S-54-HL; Saint-Gobain Plastics Inc., Akron, OH) using cyclohexanone. The...
catheter was secured to the surrounding muscle with 5.0 Vicryl suture (Ethicon Inc., Cornelia, GA). The skin was sutured with 4.0 nylon suture and exterior wounds dressed with antibiotic powder.

**Paw Withdrawal Threshold**

Mechanical allodynia following SNL was assessed by measuring PWTs using von Frey filaments (Touch Test Sensory Evaluators; Stoelting, Wood Dale, IL)\(^1\) for all animals using Dixon nonparametric statistics.\(^2\) Following at least 14 days postsurgery, PWTs were assessed; allodynia was defined as a PWT of less than 4.0 g. During assessment of drug effects on mechanical allodynia, baseline PWTs were determined 20 min before intraperitoneal or intrathecal drug injections. PWTs were then assessed 15, 60, and 120 min following intraperitoneal injection, and 60 min following intrathecal injection in SNL rats. To determine if VTA stimulation alleviated established mechanical allodynia, noncontingent electrical stimulation was delivered using similar parameters to those during self-stimulation sessions. During stimulation testing, special care was taken to prevent the occurrence of motor abnormalities that would otherwise interfere with behavioral testing. First, baseline PWTs were assessed immediately before stimulation. Next, the frequency of stimulation was set to 45 Hz and the current to 10μA; the current was then gradually increased (10μA increments) to that which was used during self-stimulation sessions (unique to each rat). Finally, PWTs were assessed in ascending order (45, 68, 103, 136, and 156 Hz), with the frequency of stimulation being gradually increased (10 Hz increments until the next scheduled test frequency) after each PWT assessment. The entire testing procedure lasted between 6 and 8 min and successfully prevented the development of motor abnormalities.

**Drugs**

Methadone hydrochloride, fentanyl hydrochloride, and oxycodone 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 μm nitrocellulose filter. Hydrodramorphone hydrochloride was purchased as a 2 mg/ml sterile solution (Hospira Inc., Lake Forest, IL). Morphine sulfate was purchased as a 15 mg/ml sterile solution (Baxter Healthcare; Deerfield, IL). Clonidine hydrochloride was purchased from Sigma-Aldrich Co. (St. Louis, MO). Adenosine was purchased as a 3 mg/ml sterile solution (Fujsawa USA Inc., Deerfield, IL). Gabapentin was purchased from Tocris Bioscience (Ellisville, MO). All drugs were diluted using 0.9% (wt/vol) saline, pH 7.4.

**Intracranial Self-stimulation**

**Apparatus.** An operant chamber with a lever 5 cm above a grid bar floor, stimulus lamp 2 cm above the lever, and tone generator was used (Med Associates Inc., St. Albans, VT). The operant chamber was housed within a sound- and light-attenuating enclosure containing a houselight and ventilation fan. An ICSS stimulator controlled by a computer software program (Med Associates Inc.) that controlled all stimulation parameters and data collection was located outside of the enclosure. A 2-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.** Following at least 14 days postsurgery, rats were trained to lever press for brain stimulation as previously described.\(^1\) A stimulus light located above the lever indicated stimulus availability. Lever presses produced a 0.5-s train of rectangular alternating cathodal and anodal pulses (0.1-ms pulse durations); during stimulation the stimulus light turned off, the houselight turned on, and a tone was sounded. Responding during the 0.5-s stimulation period resulted in no further stimulation and was not recorded.

During initial acquisition sessions the frequency was held constant (156 Hz) and the intensity was adjusted by the experimenter to determine the lowest intensity that maintained high rates of responding (more than 40 responses/min). Once responding was established, frequency-response curves were generated. These 2-h sessions consisted of six 10-min components, which were further broken down into ten 1-min trials. Each 60-s trial consisted of a 5-s period during which five noncontingent stimulations were delivered, and finally a 50-s period in which lever presses resulted in stimulation and were recorded. During these sessions the intensity remained the same (unique to each animal) and 10 frequencies (156–45 Hz, 0.06 log increments) corresponding to each trial were presented in descending order. A 1-h timeout period between the third and fourth components permitted drug injections during test sessions. During test sessions when fentanyl was administered, the timeout period was reduced to 15 min. At the beginning of the timeout period rats received 1 ml/kg intraperitoneal injections of saline (0.9% wt/vol), morphine (3 mg/kg), methadone (0.3–6 mg/kg), fentanyl (0.01–0.1 mg/kg), hydromorphone (0.03–1 mg/kg), or oxycodone (0.3–3 mg/kg); when testing intrathecal drugs, rats received 20 μl (5 μl drug followed by 15 μl saline flush) intrathecal injections of saline (0.9% wt/vol), clonidine (3 and 10 μg), adenosine (30 μg) or gabapentin (100 μg). For data analysis, the two components preceding drug injection (two and three) and the three components following drug injection (four, five, and six) were averaged and compared using Prism software (sigmoidal-dose response, variable slope; Graph Pad, La Jolla, CA). All test sessions were separated by at least 1 day. Saline was administered to each animal first, followed by subsequent administrations of methadone, fentanyl, hydromorphone, and oxycodone; for spinal analgesic testing, rats were initially tested with intraperitoneal morphine (3 mg/kg) first, followed by intrathecal administration of clonidine, adenosine, and gabapentin. Preliminary experiments...
determined the highest doses of each opioid that could be delivered without decreasing maximum response rates for VTA ICSS (data not shown in Results). All test sessions were performed between 1 and 4 months after surgery, and animals were added when needed because of attrition from electrode loss or decreased responding for VTA ICSS over time.

**Histology**

Rats were sacrificed by carbon dioxide asphyxiation. Brains were rapidly removed and frozen in isopentane (−35°C) and were stored at −°C. Coronal sections (25 μm) around the electrode tract were obtained using a cryostat to confirm electrode placement within the VTA (fig. 1).

**Data Analysis**

Data for PWTs was analyzed using a two-way ANOVA with drug dose and time after infusion serving as the independent variables. The EF50 (frequency at which rats emitted 50% of maximal responding) and maximum response rate for VTA ICSS was calculated using Prism software (sigmoidal-dose response, variable slope; Graph Pad, San Diego, CA). The effect of drug treatment on VTA ICSS was analyzed using a one-way ANOVA with drug dose serving as the independent variables and \( \text{EF50 (EF50 before injection, EF50 after injection)} \) or maximal response rates serving as the dependent measures. *Post hoc* analyses within the control or SNL groups were made using Dunnett’s *t* test for multiple comparisons with saline injection serving as control. A two-tailed \( P \) value of 0.05 \( \pm \) was considered statistically significant. All statistical analyses were performed using JMP software (version 5.0.1a; SAS Institute Inc., Cary, NC).

**Results**

**Effects of SNL and Morphine on VTA ICSS**

Consistent with previous results, the EF50 and maximum response rate for VTA ICSS was similar for control and SNL rats (fig. 2A). The EF50 for VTA ICSS in control rats was 100.2 (2.7) Hz (mean ± SEM), and for SNL rats was 100.2 (2.7) Hz \( [F(1,16) = 0, P = 1.0] \). The maximum response rate was 43.2 (2.3) and 39.9 (2.4) responses/component for control and SNL rats, respectively \( [F(1,16) = 1.0, P = 0.3] \). The stimulus intensities used for ICSS also did not differ between control (168 ± 20 μA) and SNL (144 ± 22 μA) rats \([F(1,16) = 0.6, P = 0.4]\).

Also consistent with previous data, morphine (3 mg/kg, intraperitoneal) in control rats produced a leftward shift in the frequency-response curve for VTA ICSS, reducing the EF50 from 93.3 (2.4) Hz [mean(SEM)] to 84.2 (3.4) Hz \([F(1,13) = 4.8, P = 0.048]\) (fig. 2B). Morphine had no
effect on VTA ICSS in SNL rats, however, as shown previously [F(1,13) = 0.8, P = 0.4] (fig. 2C). Saline administration had no effect on the EF50 for VTA ICSS in either control [F(1,13) = 0.2, P = 0.6] or SNL [F(1,13) = 0.0004, P = 0.98] rats. Neither saline nor morphine altered maximum response rate for VTA ICSS in either the control [F(1,13) = 0.0007, P = 0.98; F(1,13) = 0.08, P = 0.8, respectively] or SNL [F(1,13) = 1.5, P = 0.2; F(1,13) = 0.01, P = 0.9, respectively] rats.

Effects of Opioids on VTA ICSS in Control and SNL Rats

**Fentanyl.** Fentanyl shifted the frequency-response curves to the left in both control and SNL rats. The effect of fentanyl on ΔEF50 in control rats was dose-dependent [F(4,36) = 15.6, P < 0.0001], with all doses greater than 0.01 mg/kg producing a significant leftward shift in the frequency-response curve (P ≤ 0.05) (fig. 3). The effect of fentanyl on ΔEF50 was also dose-dependent in SNL rats [F(4,35) = 8.0, P = 0.001] with only the two highest doses given (0.06 and 0.1 mg/kg) producing a significant shift in the frequency-response curve compared with saline (P ≤ 0.05) (fig. 3). Fentanyl did not alter the maximum response rate at any dose in either control [F(4,36) = 0.7, P = 0.6] or SNL [F(4,35) = 1.8, P = 0.2] rats.

**Methadone.** Methadone significantly shifted the frequency-response curves for VTA ICSS to the left, in a manner similar to fentanyl, in both control and SNL rats. The effect of methadone on ΔEF50 was dose-dependent in control rats [F(4,36) = 4.7, P = 0.0004], with 3.0 and 6.0 mg/kg resulting in a ΔEF50 significantly different from saline treatment (P ≤ 0.05) (fig. 4A). The effect of methadone on ΔEF50 was also dose-dependent [F(4,39) = 4.5, P = 0.005], with only the 6.0 mg/kg dose producing a ΔEF50 significantly different from saline in SNL rats (P ≤ 0.05) (fig. 4A). As with fentanyl, methadone did not alter the maximum response rate in either control or SNL rats [control: F(4,36) = 1.4, P = 0.2; SNL: F(4,39) = 1.1, P = 0.4].

**Hydromorphone.** Hydromorphone potentiated VTA ICSS in a manner similar to that of methadone and fentanyl, shifting the frequency-response curves to the left without altering the maximum response rate in both control and SNL rats. The effect of hydromorphone on ΔEF50 was dose-dependent in control rats [F(4,36) = 8.2, P = 0.0001], with doses of 0.3 and 1.0 mg/kg producing a significantly greater effect than saline (P ≤ 0.05) (fig. 4B). Hydromorphone increased ΔEF50 values in a dose-dependent manner in SNL rats as well [F(4,35) = 5.6, P = 0.002], with only the highest dose of 1.0 mg/kg producing an effect significantly different from that produced by saline (P ≤ 0.05) (fig. 4B). Hydromorphone did not alter the maximum response rate for VTA ICSS in either control or SNL rats [saline: F(4,36) = 0.3, P = 0.9; SNL: F(4,35) = 0.2, P = 0.9].

**Oxycodone.** Oxycodone shifted the frequency-response curve for VTA ICSS in control rats in a dose-dependent manner [F(4,32) = 7.0, P = 0.0005], with doses of 1 and 3 mg/kg increasing ΔEF50 greater than saline administration (P ≤ 0.05) (fig. 4C). Oxycodone’s effect on ΔEF50 in SNL rats was not dose-dependent, however [F(4,34) = 2.1, P = 0.1] (fig. 4C). As with the other opioids tested, oxycodone did not alter the maximum response rate in control rats [F(4,32) = 0.3, P = 0.8], but did decrease the maximum response rate in SNL rats [F(4,34) = 3.0, P = 0.04], with the

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**Fig. 3.** Effects of spinal nerve ligation on fentanyl facilitation of electrical stimulation of the ventral tegmental area. (A) Reduction in EF50 (frequency at which rats emitted 50% of maximal responding) was calculated by subtracting the EF50 for the three components following drug injection (four, five, and six) from the EF50 for the two components preceding drug injection (three and four) for each dose assessed. Data shown are averages across control (n = 7) and spinal nerve ligated (n = 7) rats. Frequency-response curves before and after 0.03 mg/kg fentanyl (15-min) are shown for control (B, n = 7) and spinal nerve ligated (C, n = 7) rats. Areas marked with * are significantly different from saline treatment P ≤ 0.05. SNL = spinal nerve ligated.
rate being significantly lower following administration of 1 mg/kg compared with saline (\(P \leq 0.05\)).

**Effects of Opioids on Mechanical Allodynia in SNL Rats**

All of the opioids administered produced a reversal of mechanical allodynia in SNL rats in the range of doses that were examined using VTA ICSS (fig. 5). Administration of saline (1 ml/kg intraperitoneal) did not alter PWT at any time point \(F(3,27) = 0.5, P = 0.7\). Methadone produced a time- and dose-dependent antiallodynic effect \([\text{time}: F(3,111) = 11.2, P < 0.0001; \text{dose}: F(3,111) = 15.1, P < 0.0001]\), and there was a significant interaction between time and dose \([F(9,111) = 3.2, P = 0.002]\). Only the highest dose of methadone (6 mg/kg) produced a significant effect on PWT compared with saline \((P \leq 0.05)\). Fentanyl also produced an antiallodynic effect that was dependent upon dose \([F(3,111) = 9.9, P < 0.0001]\) and time after administration \([F(3,111) = 15.9, P < 0.0001]\), with a significant interaction between time and dose \([F(9,111) = 3.9, P = 0.0003]\). The maximum effect of fentanyl occurred 15 min after administration and both 0.06 and 0.1 mg/kg produced an effect significantly greater than saline \((P \leq 0.05)\). Hydromorphone reversed mechanical allodynia in a manner dependent on both time \([F(3,111) = 8.7, P < 0.0001]\) and dose \([F(3,111) = 9.0, P < 0.0001]\), with a significant interaction between time and dose \([F(9,111) = 2.9, P = 0.005]\). The maximum effect of hydromorphone was found 1 h after administration, with only the highest dose of 1.0 mg/kg produced an effect significantly greater than saline \((P \leq 0.05)\). Hydromorphone reversed mechanical allodynia in a matter dependant on both time \([F(3,111) = 8.7, P < 0.0001]\) and dose \([F(3,111) = 9.0, P < 0.0001]\), with a significant interaction between time and dose \([F(9,111) = 2.9, P = 0.005]\). The maximum effect of oxycodone occurred 15 min after administration and only the highest dose of 3.0 mg/kg produced an effect significantly greater than that of saline.

**Effects of Intrathecal Administration of Analgesics on VTA ICSS in SNL Rats**

Analgesics known to reverse mechanical allodynia in SNL rats following intrathecal administration did not alter the
EF50 values for VTA ICSS in SNL rats (fig. 6A). Administration of intrathecal saline did not change the EF50 for VTA ICSS in SNL rats [F(1,13) = 0.4, P = 0.6], nor did it alter the maximum response rate [F(1,13) = 0.2, P = 0.7]. Administration of intrathecal clonidine did not alter EF50 values at a dose of either 3 [F(1,13) = 0.01, P = 0.9] or 10 [F(1,13) = 2.1, P = 0.2] µg in SNL rats. The higher dose of 10 µg of clonidine decreased the maximum response rate compared with pretreatment values [F(1,13) = 7.4, P = 0.02], whereas 3 µg had no effect [F(1,13) = 2.6, P = 0.1]. Gabapentin administration was without effect on either the EF50 [F(1,13) = 0.08, P = 0.8] or maximum response rate [F(1,13) = 1.0, P = 0.3]. Adenosine administration also did not affect the frequency-response curve for VTA ICSS, producing no significant change in either the EF50 [F(1,13) = 0.1, P = 0.4] or the maximum response rate [F(1,13) = 0.5, P = 0.5].

Effect of Intrathecal Analgesics on PWT in SNL Rats

All drugs given intrathecally reversed mechanical allodynia in SNL rats with similar efficacy (fig. 6B). Saline had no effect on PWT after intrathecal administration [F(1,13) = 0.03, P = 0.9], whereas both 3 and 10 µg of clonidine significantly increased PWT compared with baseline values [3 µg: F(1,13) = 11.1, P = 0.006; 10 µg: F(1,13) = 65.3, P < 0.0001]. Comparable effects were found following intrathecal administration of 30 µg of adenosine [F(1,13) = 17.0, P = 0.001] or 100 µg of gabapentin [F(1,13) = 19.1, P = 0.001].

Effect of Electrical Stimulation of the VTA on PWT in SNL Rats

Electrical stimulation applied to the left VTA did not significantly alter PWT in SNL rats across a range of stimulation frequencies comparable with those used to maintain ICSS (45–156 Hz) [F(5,41) = 0.1, P = 0.98] (table 1). The stimulus intensity applied to the VTA in this experiment was similar to that used in the ICSS paradigm (158 ± 21 µA).

Discussion

Amid the growing prescription opioid abuse problem, there is a clear need to understand how chronic pain alters the rewarding effects of commonly abused prescription opioids. The present data indicate that SNL reduces the potency of methadone, fentanyl, and hydromorphone in producing rewarding effects, as measured using facilitation of VTA ICSS. Administration of spinal analgesics at doses that alleviated mechanical allodynia following SNL failed to facilitate VTA ICSS in SNL rats, indicating that alleviation of mechanical allodynia per se does not significantly stimulate dopaminergic pathways within the reward system. Electrical stimulation of the VTA at parameters

Table 1. Effects of Electrical Stimulation of the Ventral Tegmental Area on Mechanical Allodynia

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>PWT (g)</th>
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<tbody>
<tr>
<td>0</td>
<td>2.61 ± 0.30</td>
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<tr>
<td>45</td>
<td>2.90 ± 0.33</td>
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<tr>
<td>68</td>
<td>2.74 ± 0.33</td>
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<tr>
<td>103</td>
<td>2.92 ± 0.69</td>
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<tr>
<td>136</td>
<td>3.06 ± 0.55</td>
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<tr>
<td>156</td>
<td>2.90 ± 0.37</td>
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Data are presented as mean ± SEM (n = 7). Allodynia defined as a paw withdrawal threshold of less than 4.0 g.
PWT = paw withdrawal threshold.
similar to those experienced during VTA ICSS failed to reverse mechanical allodynia in SNL rats as well, further indicating that stimulation of reward pathways in the brain and reversal of mechanical allodynia are not interrelated. Drug effects on VTA ICSS in rats with peripheral nerve injury therefore appear to be related exclusively to abuse liability within the context of pain, unlike systemic drug self-administration or CPP, in which both analgesic and positive reinforcing effects may have significant roles in the behavioral measures.

The ability of a drug to facilitate VTA ICSS has long been suggested to be indicative of its abuse potential. Therefore, a reasonable interpretation of the current data is that the abuse potential of prescription opioids is diminished following peripheral nerve injury. Previously we reported that morphine was ineffective in facilitating VTA ICSS following nerve injury, whereas heroin was still effective but less potent.\(^\text{18}\) Heroin is an illicit drug with a relatively high abuse potential compared with morphine, attributed in part to the ability of heroin to more rapidly cross the blood brain barrier than morphine.\(^\text{21}\) where it is metabolized to 6-monoacetylmorphine,\(^\text{22}\) a metabolite that is more efficacious in stimulating \(\mu\)-opioid receptors than morphine.\(^\text{23}\) This led us to question if prescription opioids, with presumably lower abuse potential than heroin, would like morphine be ineffective in facilitating VTA ICSS following SNL. Surprisingly, each commonly abused prescription opioid, with the exception of oxycodone, shared a similar profile of reduced effects. Oxycodone was the only opioid administered was unexpected, and may be because of sedative effects. Oxycodone was the only opioid administered that decreased maximal rates of responding in SNL rats, an effect often attributed to sedative effects or general disruption of behavior.

The fact that commonly abused prescription opioids produced effects similar to heroin using the VTA ICSS procedure is interesting given previous studies evaluating opioid self-administration in SNL rats. These experiments showed that methadone and heroin retain similar self-intake rates in SNL compared with control rats, albeit at higher dose requirements, whereas self-administration rates of morphine, fentanyl, and hydromorphone are reduced following SNL compared with control subjects.\(^\text{7}\) Although there is considerable agreement between the present data on the effects of opioids on VTA ICSS and previous data on opioid self-administration in SNL rats, there are notable differences between these paradigms with fentanyl and hydromorphone. Fentanyl and hydromorphone facilitate VTA ICSS in the present study, yet were found to be poor reinforcers in self-administration experiments in SNL rats.\(^\text{7}\) These discrepancies highlight the differences between these two behavioral paradigms. The subjective effects of electrical stimulation of a discrete population of cells in the brain are undoubtedly different than those produced following systemic administration of an opioid. Drug self-administration requires an animal to evaluate many factors pertinent to the subjective effects of the drug, including drug onset, duration of action, and potential rewarding/aversive effects associated with drug intake. Drug facilitation of ICSS, on the other hand, is a much more passive approach; drug administration is experimenter-delivered and the ability of the drug to alter a previously learned behavior serves as the primary dependent measure. Studies using both paradigms indicate that opioid reward is diminished in SNL rats, providing support for the complementary use of these two paradigms in understanding the interaction between the presence of pain and opioid addiction.

Another major finding of the current work was the inability of spinal analgesics to facilitate VTA ICSS in SNL rats. Given that administration of the spinal analgesic clonidine has previously been shown to elicit rewarding effects selectively in rats with neuropathic pain,\(^\text{11,12}\) it was hypothesized that alleviation of mechanical allodynia in nerve-injured rats could stimulate brain dopamine pathways, and subsequently facilitate VTA ICSS. This was not the case, as administration of intrathecal clonidine, adenosine, and gabapentin, at doses that alleviated mechanical allodynia, failed to alter VTA ICSS. This suggests that alleviation of mechanical allodynia per se is not rewarding in the sense that it activates mesolimbic dopamine reward pathways. It also provides further support against the notion that activation of brain dopamine reflects a final pathway necessary in drug reinforcement,\(^\text{24}\) particularly in the context of pain. Previous studies assessing the rewarding effects of intrathecal clonidine using CPP\(^\text{11}\) and intrathecal self-administration\(^\text{12}\) both assessed the effects of 10 \(\mu\)g clonidine, however this dose significantly reduced maximal responding for VTA ICSS. Decreases in maximum response rates associated with VTA ICSS can be difficult to interpret, and for this reason we tested a lower dose (3 \(\mu\)g) that did not alter maximal responding or facilitate VTA ICSS, but still reversed mechanical allodynia.

In the present study we found that activation of limbic dopamine pathways by electrical stimulation of the VTA at similar parameters to those used during VTA ICSS failed to reverse established mechanical allodynia following SNL. This finding is consistent with previous reports that psycho-stimulants, which function predominantly to increase dopamine transmission, are effective in alleviating tonic (e.g., pain behaviors following formalin injection) but not phasic pain (e.g., tail-flick).\(^\text{25}\) SNL rats do not exhibit clear overt behavioral manifestations of ongoing pain, and because of this we could not test whether VTA stimulation alters ongoing pain following nerve injury.

The current data reveal that commonly abused prescription opioids stimulate classic reward pathways at similar doses to those that reverse mechanical allodynia in rats following peripheral nerve injury. It is possible that the overlap in effective doses indicates a direct relationship between opioid alleviation of allodynia and opioid stimulation of mesolimbic dopamine, such that one affects the other. This is unlikely for several reasons. We previously reported that doses of morphine that reversed mechanical allodynia did not facilitate VTA ICSS; doses of cocaine
that facilitated VTA ICSS did not alleviate mechanical allodynia in SNL rats. Also, since spinal analgesics alleviated mechanical allodynia but failed to facilitate VTA ICSS, and electrical stimulation of the VTA did not alter mechanical allodynia, it is clearly possible to alleviate hypersensitivity without stimulating the mesolimbic dopaminergic system, and vice versa in SNL rats. Therefore, the overlap in effective doses for reversing hypersensitivity and facilitating VTA ICSS by opioids is unlikely because of one affecting the other. Future studies would be helpful in this respect, by determining if the suppressive effects of nerve injury on opioid facilitation of VTA ICSS can be prevented or reversed with nonopioid treatments that prevent or reverse hypersensitivity and/or ongoing pain following nerve injury.

The current data support the notion that prescription opioids are less effective in activating brain reward pathways following peripheral nerve injury in rats, and also suggest that effects on VTA ICSS are independent of reversal of mechanical hypersensitivity. With abuse a major concern in prescribing opioids for pain, it is ideal that the rewarding effects of opioids be suppressed in chronic pain states. It should be concerning, however, that the suppressive effects of SNL on opioid facilitation of VTA ICSS generally only reduced the potency of each opioid assessed in the current study. Neuropathic pain patients often have higher opioid dose requirements than those used to treat acute nociceptive pain. In a double-blind study, opioids were shown efficacious in treating neuropathic pain; however, high doses produced significantly better reductions in reported pain intensity. High opioid dose requirements in neuropathic patients may reflect a diminished ability of opioids to either inhibit ascending noxious input or to activate brainstem-descending pain modulation circuitry following neuropathy. It is also possible that stimulation of limbic dopamine modulates neuropathic patients’ subjective pain scores, and that higher opioid dose requirements reflect decreased opioid stimulation of mesolimbic dopamine, similar to the decreased facilitation of VTA ICSS observed in SNL rats.

In conclusion, operant paradigms such as drug self-administration and VTA ICSS, along with conditioning experiments such as CPP, function as complementary preclinical tools for examining drug reinforcement mechanisms in the presence of pain. The use of all three strategies could assist in developing effective analgesics with diminished abuse potential, or in developing drugs that reduce the abuse liability of opioids without altering their analgesic properties.

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