Title

Blockade of dopamine D1 receptors in male rats disrupts morphine reward in pain naïve but not in chronic pain states

Journal

Journal of Neuroscience Research, 100(1)

ISSN

0360-4012

Authors

Grenier, Patrick
Mailhiot, Madison C
Cahill, Catherine M
et al.

Publication Date

2022

DOI

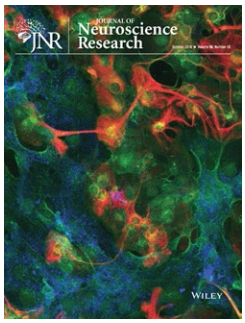
10.1002/jnr.24553

Peer reviewed

Received: 26 August 2019 | Revised: 18 October 2019 | Accepted: 22 October 2019

DOI: 10.1002/jnr.24553

RESEARCH ARTICLE



Blockade of dopamine D1 receptors in male rats disrupts morphine reward in pain naïve but not in chronic pain states

Patrick Grenier[1] | Madison C. Mailhiot[1] | Catherine M. Cahill[2,3] | Mary C. Olmstead[1,4]

[1]Department of Psychology, Queen's University, Kingston, ON, Canada

[2]Hatos Center for Neuropharmacology, Semel Institute for Neuroscience and Human Behavior, University of California Los Angeles, Los Angeles, CA, USA

[3]Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, CA, USA

[4]Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada

Correspondence
Mary C. Olmstead, Department of Psychology, Queens University, 62 Arch Street, Kingston, ON K7L 3N6, Canada.
Email: olmstead@queensu.ca

Funding information
This work was supported by an Operating Grant from the Canadian Institutes of Health Research to CMC and MCO (MOP: 123298), a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to MCO (203707), and an NSERC graduate fellowship to PG.

Abstract

The rewarding effect of opiates is mediated through dissociable neural systems in drug naïve and drug-dependent states. Neuroadaptations associated with chronic drug use are similar to those produced by chronic pain, suggesting that opiate reward could also involve distinct mechanisms in chronic pain and pain-naïve states. We tested this hypothesis by examining the effect of dopamine (DA) antagonism on morphine reward in a rat model of neuropathic pain.Neuropathic pain was induced in male Sprague-Dawley rats through chronic constriction (CCI) of the sciatic nerve; reward was assessed in the conditioned place preference (CPP) paradigm in separate groups at early (4-8 days post-surgery) and late (11-15 days post-surgery) phases of neuropathic pain. Minimal effective doses of morphine that produced a CPP in early and late phases of neuropathic pain were 6 mg/kg and 2 mg/kg respectively. The DA D1 receptor antagonist, SCH23390, blocked a morphine CPP in sham, but not CCI, rats at a higher dose (0.5 mg/kg), but had no effect at a lower dose (0.1 mg/kg). The DA D2 receptor antagonist, eticlopride (0.1 and 0.5 mg/kg), had no effect on a morphine CPP in sham or CCI rats, either in early or late phases of neuropathic pain. In the CPP paradigm, morphine reward involves DA D1 mechanisms in pain-naïve but not chronic pain states. This could reflect increased sensitivity to drug effects in pain versus no pain conditions and/or differential mediation of opiate reward in these two states.

KEYWORDS

addiction, analgesia, antinociception, aversion, chronic pain, dependence, opioid, reward

1 | INTRODUCTION

Chronic pain, which affects 20%–30% of the population (Blyth et al., 2001; Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006; Johannes, Le, Zhou, Johnston, & Dworkin, 2010; Schopflocher, Taenzer, & Jovey, 2011), is commonly treated with opioid analgesics. Although effective in the short-term, long-term use of these drugs can lead to reduced analgesia (tolerance) (Christie, 2008), hyperalgesia (Mao, 2002), and addiction (Contet, Kieffer, & Befort, 2004). Diversion of prescription opioids exacerbates these problems and has contributed to an epidemic of drug overdose and death throughout North America and many parts of Europe (Volkow & McLellan, 2016). Thus, there is a pressing need to develop effective

Patrick Grenier and Madison C. Mailhiot contributed equally to this work.

Edited by Tuan Trang, University of Calgary. Statistical Editor: David McArthur.

The peer review history for this article is available at https://publons.com/publon/10.1002/jnr.24553.

treatments for chronic pain with low abuse potential (Evans & Cahill, 2016).

A major challenge in this endeavor is that chronic pain, itself, alters brain reward systems (Taylor, Becker, Schweinhardt, & Cahill, 2016; Trang et al., 2015) that are associated with addiction (Volkow, Koob, & McLellan, 2016; Wise, 2008). For example, induction of neuropathic pain in rats inhibits dopamine (DA) transmission from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), with dysfunction emerging as animals transition from acute to chronic pain (Taylor, Castonguay, & Taylor, 2015). Human imaging studies confirm that chronic pain is associated with disruptions in mesolimbic DA function (Hagelberg, Forssell, & Aalto, 2003; Hagelberg, Forssell, & Rinne, 2003; Wood, Schweinhardt, & Jaeger, 2007); these alterations may explain differences in neural mechanisms that mediate morphine reward in chronic pain and pain-free states (Cahill et al., 2013; Ozaki, Narita, & Narita, 2002). A comparable dissociation has been observed in drug-naïve and drug-dependent rats (Bechara, Harrington, Nader, & Kooy, 1992), with the rewarding effect of morphine mediated by DA D1 and D2 receptors in opiate-naive and opiate-dependent states, respectively (Lintas, Chi, & Lauzon, 2011). Given that both chronic pain and repeated drug exposure alter mesolimbic DA function, the same dissociation may be evident in pain and pain-free conditions.

This study continued our previous investigation into mechanisms of morphine reward in pain-naive and chronic pain states (Cahill et al., 2013), specifically testing whether DA D1 and D2 mechanisms have distinct roles in these two processes. Reward was assessed using the conditioned place preference (CPP) paradigm and chronic pain was induced using a chronic constriction injury (CCI) model of neuropathic pain (Bennett & Xie, 1988). Neuropathic pain is characterized by allodynia (pain responses to previously neutral stimuli) and hyperalgesia (heightened or sensitized pain responses), which is presented in rats as a change in threshold to sensory stimuli that increases in intensity across an 8 to 12-day period in rats. A time-dependent change in sensory hypersensitivities reflect neuroadaptations following nerve injury, with distinct biological processes mediating pain responses in early and late phases of NP pain (Asaoka, Kato, Ide, Amano, & Minami, 2018; Kato, Ide, & Minami, 2016; Kleinschnitz, Brinkhoff, Zelenka, Sommer, & Stoll, 2004). We, therefore, examined the rewarding effect of morphine at two separate time points following CCI surgery and used a CPP protocol that ensured conditioning and CPP testing did not overlap in the two groups. We assessed the role of DAergic receptors in morphine reward by administrating the D1 antagonist, SCH23390, or D2 antagonist, eticlopride, prior to conditioning using doses that block a morphine CPP in opiate-naïve and opiate-dependent states (Lintas et al., 2011).

Significance

This study is a continuation of our long-term research program examining the relationship between the analgesic and rewarding effects of abused drugs. Using a clinically relevant model of chronic pain, we demonstrate that blockade of dopamine D1 receptors reduces opiate reward, but *only* in pain-naïve states. These results add to the growing literature on alterations to reward system function in chronic pain. Our study is timely given the current opioid epidemic and the pressing need to develop new therapeutic compounds for chronic pain with minimal abuse potential.

2 | MATERIALS AND METHODS

2.1 | Subjects

Three hundred and three male Sprague-Dawley rats, weighing 250–350 g (postnatal day 51–58) were transported from the supplier (Charles River Laboratories, Montreal, QC) on a Monday morning in boxes containing six animals each. The following morning, they were moved to pair-housing in polycarbonate cages (45.5 × 24 × 21 cm) with beta chip bedding (Northeastern Products Corp, Warrensburg, NY) and a black, plastic tube (PVC piping ~7.5 cm diameter) provided as enrichment. Cages were kept in a climate-controlled room (21°C) on a reverse 12:12 hr light cycle (lights off at 07:00 hr), with humidity levels ranging from 20% to 70% across seasons. Animals had *ad libitum* access to standard rat chow (Labdiet, PMI Nutrition International, Brentwood, MO) and water in their home cage; behavioral testing was conducted during the dark cycle. Animals habituated to the facility, including handling, for a minimum of 3 days prior to the start of surgical procedures. Cages were cleaned twice per week and rats were handled daily, exclusively, by the researcher conducting the experiment. All experiments were conducted in accordance with the guidelines for the ethical use of animals outlined by the Canadian Council on Animal Care and the experiments were approved by the Queen's University Animal Care Committee.

2.2 | Apparatus

The CPP apparatus was made of plexiglass and consisted of two large compartments (46 × 46 × 30 cm) connected by a tunnel (19 × 38 × 30 cm). The two large compartments differed in wall color (black and white stripes or solid white) and floor texture (striated or bumpy). The tunnel walls were clear and the floor was made of sheet metal, spray painted with a matte black finish. Guillotine doors, that could be raised or lowered, separated the tunnel from each compartment. Cameras were mounted directly above each set of boxes so that the rats' movement throughout the entire compartment could be monitored and recorded. Movement was tracked using the video tracking software EthoVisonXT (Noldus Information Technology version 9.0.720 Wageningen, The Netherlands). Data were analyzed using IBMS SPSS Statistics (Version 25) and Microsoft Office Excel (2016); figures were produced using Prism 7 for Windows (Version 7.03).

2.3 | Surgery

Rats were randomly assigned to neuropathic or sham surgery groups; neuropathic pain was induced through CCI of the sciatic

nerve (Bennett & Xie, 1988). Prior to surgery, animals received liquid acetaminophen (0.6 ml of 32 mg/L dose, orally) and were then anesthetised under isoflurane (5 L/min induction, 2–3 L/min maintenance). All animals received 5 ml of Lactated Ringer's Solution by subcutaneous (s.c.) injection. The left hind limb was shaved and cleaned with alcohol and Betadine solution. An incision was made on the hind limb and the muscle layers were bluntly dissected with scissors to expose the sciatic nerve. Using 4-0 chromic gut suture thread, four ligatures were tied loosely around the sciatic nerve, and the muscle and skin were sutured with Monocryl 3-0 thread. Animals receiving sham surgeries had skin and blunt muscle dissection with no manipulation of the sciatic nerve. Following surgery, all animals were pair housed in a separate recovery room for 3 days, then returned to their original colony housing.

2.4 | Drugs

Morphine sulfate was supplied by Sandoz Canada Inc. (Boucherville, QC); naloxone HCl dithydrate, SCH23390 (DA D1 receptor antagonist), and eticlopride (DA D2 receptor antagonist) were purchased from Sigma-Aldrich (Milwaukee, WI). All drugs were dissolved in 0.9% physiological sterile saline, which constituted the vehicle injections (1 ml/kg).

2.5 | Behavioral procedures

2.5.1 | Establishing a morphine CPP in early and late phases of chronic pain

Separate groups of CCI rats were tested for a morphine CPP in early (n = 8) and late phases (n = 10) of chronic pain to establish doses that produced a CPP in each condition (post-surgery days 4–8 and 11–15, respectively). Prior to the first CPP session, CCI and sham rats were placed in the tunnel and had access to all three compartments for 30 min (habituation). During habituation, there was no bias to one conditioning compartment or the other. Over the next 4 days (day 4–7 or 11–14 post-surgery), rats received two conditioning sessions per day (separated by ~9 hr) in which they were injected with morphine or vehicle and confined to one of the large compartments for 30 min. The assignment of drug-paired compartment and order of conditioning sessions were counterbalanced within groups.

The lowest dose tested (2 mg/kg) produced a CPP in a late [$t(9) = 8.413$, $p < 0.001$], but not an early [$t(7) = 1.385$, $p = 0.211$], phase of chronic pain. Increasing the dose to 4 mg/kg (n = 8) was also ineffective [$t(7) = 1.562$, $p = 0.164$]; 6 mg/kg (n = 8) produced a CPP in early phase chronic pain [$t(7) = 6.489$, $p < 0.001$]. Thus, in all subsequent experiments, rats tested for a CPP during early and late phases of chronic pain were injected with 6 and 2 mg/kg morphine (s.c.), respectively. Data from the effective doses in this initial experiment are included as controls in subsequent analyses.

2.5.2 | Withdrawal

To confirm that the CPP dosing protocol did not induce physical dependence, a separate group of surgery-naïve rats (n = 8) was tested for opiate withdrawal symptoms before and after morphine injections of the higher dose (6 mg/kg s.c. per day × 4 days). Baseline (BL) behavior was assessed in an open field (46 × 46 × 30 cm) 24 hr prior to first injection; naloxone (1 mg/kg, s.c.) was administered 2 hr after the last morphine injection and naloxone-precipitated withdrawal was determined for 30 min following naloxone injection. Behavior was video recorded and withdrawal symptoms were scored according to an established scale (Schulteis, Markou, Gold, Stinus, & Koob, 1994) that includes head shakes, wet dog shakes, teeth chattering, paw tremors, diarrhea, and mouthing. The number of times each behavior occurred in 10-min bins was calculated over the 30 min. There was no difference in withdrawal scores before or after morphine treatment; mean counts of head shakes, wet dog shakes, teeth chattering, paw tremors, and diarrhea were all <1 across both 30-min sessions (see Table 1).

2.5.3 | Effects of DA antagonism on a morphine CPP

The effects of DA antagonism on the development of a morphine CPP were assessed in separate groups of CCI and sham-lesioned rats in early and last phases of chronic pain. The experimental timeline for each drug and surgery group is shown in Figure 1; subject numbers are presented in Table 2 (total n = 197). The two CCI control groups (i.e., no DA antagonist) for the early phase came from the experiment establishing morphine dose elicits a CPP in an early (6 mg/kg)

TABLE 1 Mean counts of withdrawal behaviors, prior to and following morphine treatment

	Pre-morphine			Post-morphine		
Withdrawal measure	**0–10 min**	**10–20 min**	**20–30 min**	**0–10 min**	**10–20 min**	**20–30 min**
Head shakes	0	0.13	0.13	0.38	0	0
Wet dog shakes	0	0	0	0	0	0.13
Teeth chattering	0	0	0	0	0	0
Paw tremors	0	0	0	0	0.25	0
Diarrhea	0	0	0	0	0	0
Mouthing	0.13	0	0.38	0	0.25	0

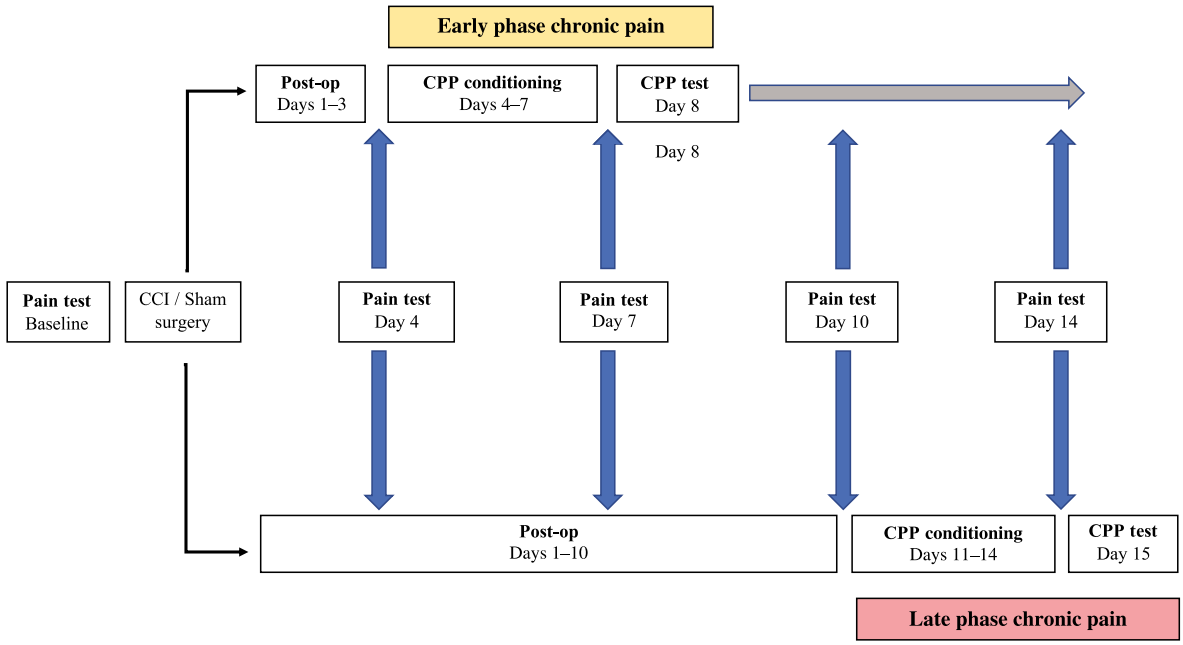


FIGURE 1 Experimental timeline for nociceptive and conditioned place preference (CPP) testing. Separate groups of chronic constriction injury (CCI) and sham-lesioned rats were tested for a morphine CPP in early or late phases of chronic pain (conditioning 4–7 and 11–14 days post-surgery). All animals were tested for thermal and mechanical nociceptive responses 1 day prior to surgery (baseline) as well as 4, 7, 10, and 14 days post-surgery

TABLE 2 Subject numbers for each surgery and drug group

			D1 antagonism		D2 antagonism	
			SCH23390		Eticlopride	
Pain phase	Surgery group	Control	0.1 mg/kg	0.5 mg/kg	0.1 mg/kg	0.5 mg/kg
Early	CCI	*n* = 8	*n* = 11	*n* = 12	*n* = 11	*n* = 10
	Sham	*n* = 8	*n* = 10	*n* = 9	*n* = 9	*n* = 9
Late	CCI	*n* = 10	*n* = 11	*n* = 11	*n* = 10	*n* = 12
	Sham	*n* = 10	*n* = 8	*n* = 9	*n* = 10	*n* = 9

phase of chronic pain (described above). The CCI control group for the late phase of chronic pain came from a group that received an injection of saline (1 ml/kg) 10 min prior to receiving morphine (2 mg/kg s.c.). The role of DA D1 and D2 antagonism on morphine reward in early and late phases of chronic pain was assessed by administering SCH23390 or eticlopride (0.1, or 0.5 mg/kg intraperitoneally, i.p.) 10 min prior to each morphine injection during conditioning. On test day (days 8 and 15 post-surgery), rats were placed in the tunnel and the amount of time spent in each compartment was recorded over 30 min. Half of the animals in each group were tested in the morning and half in the afternoon.

2.5.4 | Effects of DA antagonism on nociceptive responses

All CCI and sham animals underwent nociceptive testing for mechanical allodynia, with testing conducted 1 day prior to surgery (BL) and then 4, 7, 10, and 14 days post-surgery. Figure 1 shows the relationship between the timing of nociceptive testing and CPP conditioning. When the two protocols overlapped, nociceptive testing was conducted prior to the first (day 4 post-surgery) or after the last (days 7 and 14 post-surgery) conditioning session. Nociceptive testing on days 7 and 14 occurred either ~3.5 or ~12.5 hr after a drug injection (saline or morphine with or without DA antagonist), depending on whether animals received drug prior to the morning or afternoon session. The peak analgesic effect of morphine (s.c.) in rats is 15–30 min with an antinociceptive half-life of ~2 hr so sensory testing occurred beyond this window (Berkotitz, Cerreta, & Spector, 1974). We also confirmed that there was no significant difference in nociceptive withdrawal scores on days 7 and 14 for animals receiving drug prior to morning or afternoon conditioning sessions (*ps* > 0.05).

All animals had 30–45 min habituation in the procedure room prior to the initiation of sensory testing. Mechanical allodynia was measured using von Frey filaments. First, animals were placed on top of an elevated wire grid for a 10-min habituation period. Then, a 12 g (5.07) filament (Stoelting, Wood Dale, IL) was applied to the bottom

of the ipsilateral hind paw and the number of withdrawals per 10 applications was recorded for each animal. All CCI rats exhibited a sustained reduction in von Frey threshold across the duration of an experiment, and no data were excluded from subsequent analyses.

2.5.5 | Effects of DA antagonism on place conditioning

To determine whether D1 receptor antagonism may affect motivation, CCI and sham rats (n = 8 each) were tested in the CPP paradigm following conditioning with SCH23390 (0.5 mg/kg i.p.) and saline (1 ml/mg s.c.), using the protocol described previously (i.e., no morphine was administered). The experiment was conducted during late phase chronic pain (i.e., days 11–14 post-surgery) to coincide with peak nociceptive responses in the CCI model.

2.5.6 | Effects of DA antagonism on morphine-induced antinociception

To determine whether DA D1 antagonism blocked a morphine CPP by reducing sensory nociception, separate groups of CCI and sham-lesioned rats underwent nociceptive testing in early and late phases of chronic pain following injections of either saline (1 ml/kg i.p) or SCH233390 (0.5 mg/kg i.p.) administered 10 min prior to morphine (6 or 2 mg/kg) (n = 6 each; total n = 48). Each animal was tested on the day prior to surgery (drug-free) and then on four consecutive days that coincided with post-surgery days and times of CPP conditioning sessions (i.e., days 4–7 and 11–14 in early and late phase chronic pain). Sensory threshold testing was initiated 10 min following the injection of morphine and was completed within 30 min.

As described previously, mechanical withdrawal thresholds were assessed as the number of withdrawals per 10 applications of a 12-g filament. Ten minutes later, animals were tested for thermal hyperalgesia using a tail flick analgesiometer (IITC Life Science Inc., Woodland Hills, CA) that projects a beam of light onto the rat's tail. The distal 5-cm portion of the tail was blackened with permanent marker to facilitate heat absorption. Heat intensity was adjusted to elicit tail flick latencies between 2 and 3 s with a 10-s cut off period. The tail was placed under the heat source with the rat gently wrapped in a towel; the time to flick the tail from the heat source was recorded manually. The test was repeated 10–15 min later and the average of the two latency scores was used.

2.6 | Statistical analyses

Data from CPP experiments were analyzed using four separate three-way factorial analyses of variances (ANOVAs) for both early (SCH23390, eticlopride) and late (SCH23390, eticlopride) phases of chronic pain. Surgery condition (CCI, sham), DA antagonist dose (0, 0.1, 0.5 mg/kg), and side of CPP apparatus (drug-paired, saline-paired) were included as between-subjects factors. A priori planned orthogonal comparisons were calculated to compare the amount of time spent in drug- and saline-paired compartments during testing. These a priori tests are used to analyze a limited number of predicted hypotheses. The primary advantage of conducting planned orthogonal contrasts is that these minimize the number of comparisons to those of interest, based on specific hypotheses (e.g., CCI rats administered low-dose SCH will spend more time in the morphine-paired compartment than the saline-paired compartment). Because each comparison is independent and tests a unique hypothesis, it can be carried out regardless of the outcome of the overall ANOVA and no correction is made for using multiple tests. As such, a 5% risk of type I error is accepted for each comparison. Planned comparisons were used rather than an overall three-way ANOVA with post hoc comparisons because main effects do not yield any meaningful information in this analysis (e.g., the factor "drug" reflects a comparison of the combined time in drug- and saline-paired compartments across surgery groups). In addition, a three-way ANOVA on our data would yield 28 post hoc comparisons, requiring a correction (and justification) for family-wise error. More importantly, the only post hoc comparisons that would be meaningful in this analysis are exactly those that we identify in the planned comparisons analysis: time spent in drug- versus saline-paired compartment for each group.

Using planned orthogonal comparison to analyze CPP data eliminates the possibility of comparing group differences in the magnitude of a CPP. To deal with this issue, we created the variable, difference score, which was calculated as time spent in morphine-paired minus saline-paired compartments for each group. These difference scores were compared between surgery groups in the early and late phases of chronic pain experiments using independent samples t tests. In cases where assumptions were violated, we used a bootstrapped independent samples t test (number of samples = 10,000), which is a non-parametric alternative. This tests samples with replacement to empirically derive a null hypothesis distribution of the mean differences between groups. If the overall mean difference between groups falls outside of the 95% percentile corrected confidence interval (CI) of the differences, the null hypothesis is rejected.

Outliers, normality, and homogeneity of variances were assessed using boxplots, the Shapiro–Wilk test, and Levene's test, respectively. Any violations are presented in the Results section. Outliers were not excluded from further data analysis. Additionally, we have not corrected for non-normality or violations of homogeneity of variance, as ANOVAs are relatively robust to these violations, particularly when samples sizes are approximately equal (Glass, Peckham, & Sanders, 1972).

Data from mechanical and thermal nociceptive tests were analyzed using separate mixed-model repeated measures ANOVAs, which assess statistically significant differences between means of independent groups. In this analysis, surgery (CCI vs. sham) and drug dose were included as between-subject factors and day was included as a within-subjects (repeated) factor. Tukey's post hoc analysis was performed for multiple comparisons. Sphericity was assessed using Mauchly's test and violations were corrected using a

Greenhouse–Geisser correction. Levene's *F* test was used to examine the assumptions of homogeneity of variance and Shapiro–Wilk test assessed normality. We lacked sufficient prior data for an a priori power analysis; thus we based our sample size on previous studies using similar methods (Cahill et al., 2013).

3 | RESULTS

3.1 | Effects of DA antagonism on a morphine CPP

3.1.1 | Morphine CPP in early phase chronic pain

As shown in Figure 2a and confirmed by a series of planned orthogonal comparisons, morphine (6 mg/kg s.c.) produced a robust CPP in both sham, $t(104) = 4.048$, $p < 0.001$, and CCI, $t(104) = 8.489$, $p < 0.001$, rats during the early phase of chronic pain. The magnitude of the effect was larger in CCI rats, but did not reach a statistical significance (bootstrapped independent samples *t* test, $M_{diff} = -472.525$, 95% $CI_{diff} = -980.779–35.730$, $p > 0.05$). A morphine CPP was blocked in sham rats by the higher dose of SCH23390, $t(104) = 1.008$, $p = 0.315$, but remained intact in all other groups [sham 0.1 mg/kg: $t(104) = 2.392$, $p = 0.019$; CCI 0.1 mg/kg $t(104) = 4.446$, $p < 0.001$; CCI 0.5 mg/kg: $t(104) = 6.130$, $p < 0.001$] (Figure 2b). For this ANOVA, there were three outliers that were greater than 1.5 times the interquartile range (IQR) (CCI, 0 mg/kg, drug and saline-paired sides; sham, 0.1 mg/kg, drug-paired side). Normality was violated in one group (CCI, 0 mg/kg, saline-paired side), Shapiro–Wilk's $W = 0.737$, $df = 8$, $p = 0.006$. The assumption of homogeneity of variance was also violated, $p = 0.039$. We used a bootstrapped independent samples *t* test to compare the magnitude of morphine CPP in CCI and sham rats because there was one outlier greater than 1.5 times the IQR in the CCI group.

Eticlopride had no effect on a morphine CPP in any of the groups [sham 0.1 mg/kg: $t(98) = 5.414$, $p < 0.001$; CCI 0.1 mg/kg: $t(98) = 7.338$, $p < 0.001$; sham 0.5 mg/kg $t(98) = 3.801$, $p < 0.001$; CCI 0.5 mg/kg: $t(98) = 6.305$, $p < 0.001$] (Figure 2c). In this ANOVA, there were four outliers that were greater than 1.5 times the IQR (CCI, 0 mg/kg, drug- and saline-paired sides; 2 × sham, 0.1 mg/kg, drug-paired side). Normality was violated in one group (CCI, 0 mg/kg, saline-paired side), Shapiro–Wilk = 0.737, $df = 8$, $p = 0.006$.

3.1.2 | Morphine CPP in late phase chronic pain

Morphine (2 mg/kg s.c.) produced a CPP during late phases of chronic pain in both sham, $t(106) = 6.158$, $p < .001$, and CCI, $t(106) = 8.285$, $p < 0.001$, rats (Figure 3a). Again, the size of the CPP was larger in CCI than sham rats, although the effect did not reach statistical significance (bootstrapped independent samples *t* test, $M_{diff} = -198.243$, 95% $CI_{diff} = -479.149–82.672$, $p > 0.05$). As previously, the higher dose of SCH23390 blocked the effect, but only in sham rats [sham 0.1 mg/kg: $t(106) = 2.396$, $p = 0.018$; sham 0.5 mg/kg: $t(106) = 0.187$, $p = 0.851$; CCI 0.1 mg/kg $t(106) = 5.729$, $p < 0.001$; CCI 0.5 mg/kg: $t(106) = 3.890$, $p < 0.001$] (Figure 3b).

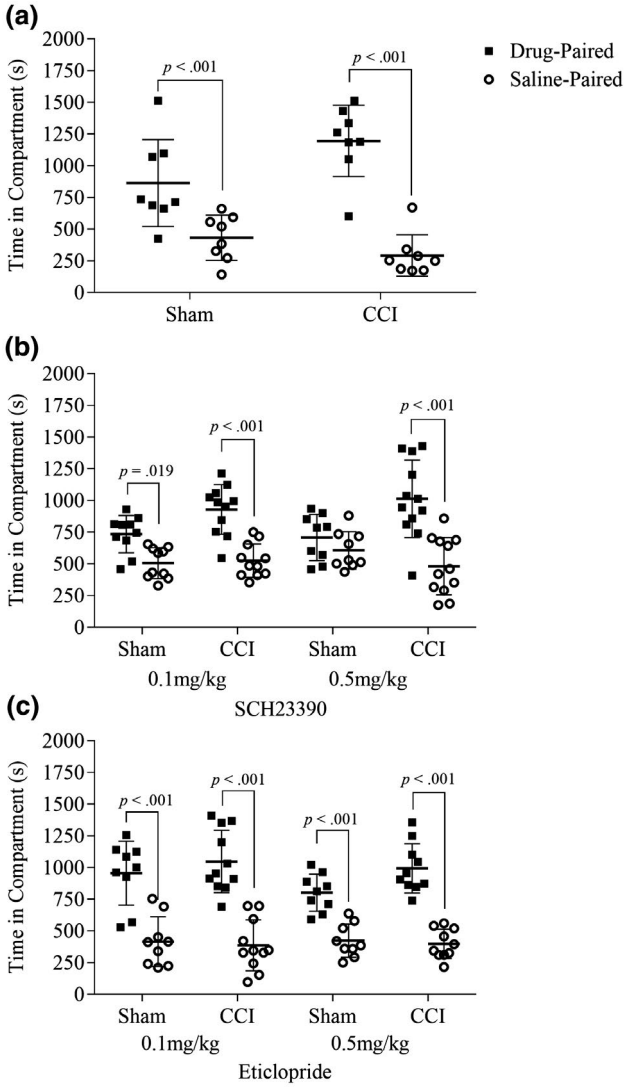


FIGURE 2 Effects of dopamine (DA) antagonism on a conditioned place preference to morphine in an early phase of chronic pain (days 4–8 post-surgery). Bars represent mean (±*SD*) time spent in drug- and saline-paired compartments over a 30-min drug-free test. Separate groups of chronic constriction injury (CCI) and sham-lesioned rats were conditioned with morphine (6 mg/kg) (a), morphine plus the DA D1 antagonist, SCH233390 (b), or morphine plus the DA D2 antagonist, eticlopride (c). Antagonist drugs were administered 10 min prior to morphine at doses of 0.1 or 0.5 mg/kg. Data points represent individual rats

There were three outliers in this ANOVA—two were >1.5 times the IQR (sham, 0 mg/kg, saline-paired side; sham, 0.5 mg/kg, drug-paired side) and one was >3 times the IQR (sham, 0.5 mg/kg, drug-paired side). Normality was violated in three groups (sham, 0 mg/kg, saline-paired: Shapiro–Wilk = 0.814, $df = 10$, $p = 0.021$; sham, 0.1 mg/kg, drug-paired: Shapiro–Wilk's $W = 0.820$, $df = 8$, $p = 0.047$; sham, 0.5 mg/kg, drug-paired: Shapiro–Wilk's $W = 0.760$, $df = 9$, $p = 0.007$). A bootstrapped independent samples *t* test was used to compare the magnitude of morphine CPP in CCI and sham rats

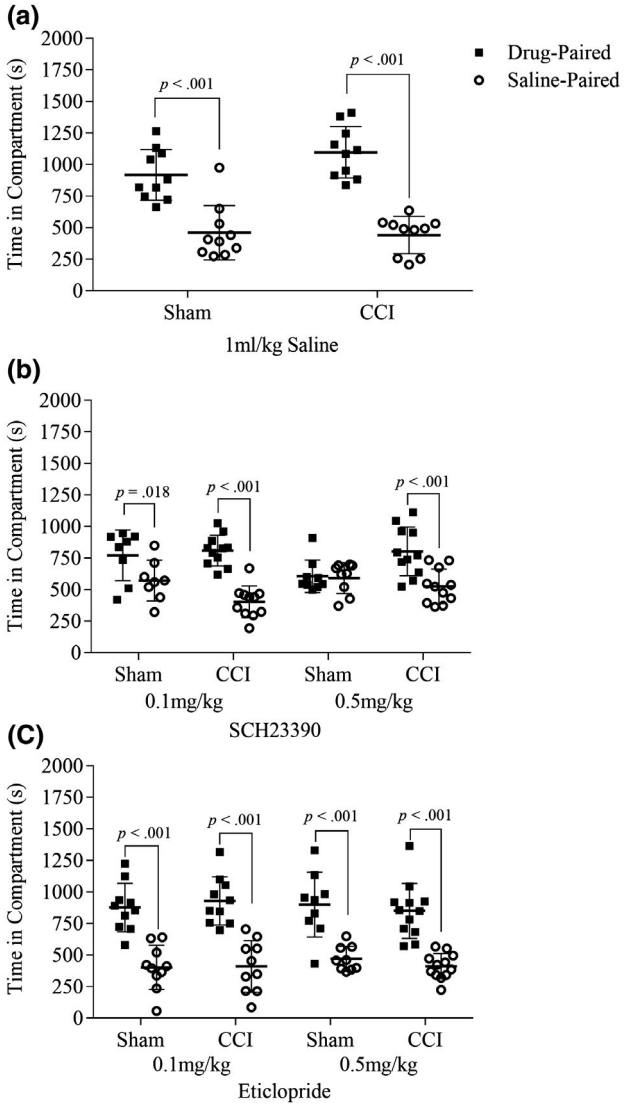


FIGURE 3 Effects of dopamine (DA) antagonism on a conditioned place preference to morphine in a late phase of chronic pain (days 11–15 post-surgery). Bars represent mean (±*SD*) time spent in drug- and saline-paired compartments over a 30-min drug-free test. Separate groups of chronic constriction injury (CCI) and sham-lesioned rats were conditioned with morphine (2 mg/kg) plus saline (a), morphine (2 mg/kg) plus the DA D1 antagonist, SCH233390 (b), or morphine (2 mg/kg) plus the DA D2 antagonist, eticlopride (c). Antagonist drugs were administered 10 min prior to morphine at doses of 0.1 or 0.5 mg/kg. Data points represent individual rats

because there was one outlier greater than 1.5 times the IQR in the sham group.

As with early phases of chronic pain, eticlopride had no effect on a morphine CPP [sham 0.1 mg/kg: $t(110) = 5.644$, $p < 0.001$; CCI 0.1 mg/kg: $t(110) = 6.163$, $p < 0.001$; sham 0.5 mg/kg $t(110) = 4.828$, $p < 0.001$; CCI 0.5 mg/kg: $t(110) = 5.709$, $p < 0.001$] (Figure 3c). Five outliers were detected as being greater than 1.5 times the IQR (CCI, 0.5 mg/kg, drug-paired side; sham, 0 mg/kg, drug-paired side; sham, 0.1 mg/kg, saline-paired side; 2 × sham, 0.5 mg/kg, drug-paired side). Normality was violated in one group (sham, 0 mg/kg, saline-paired: Shapiro–Wilk = 0.814, $df = 10$, $p = 0.021$).

3.2 | Effects of DA antagonism on nociceptive responses

3.2.1 | Nociceptive responses in early phase chronic pain

Repeated measures ANOVAs and data presented in Figure 4 confirm that CCI surgery induced mechanical allodynia, with mechanical stimulus responses increasing across testing in CCI, but not sham, rats treated with morphine plus SCH23390, surgery × day interaction: $F(20, 208) = 35.058$, $n = 58$, $p < 0.001$ (Figure 4a) and morphine plus eticlopride, $F(20, 196) = 23.456$, $n = 55$, $p < 0.001$ (Figure 4b). All testing was conducted in a drug-free state. In addition to significant differences in withdrawal scores of CCI and sham animals on all post-surgery testing days, post hoc tests revealed lower mechanical responses on day 14 in CCI animals treated with either SCH23390 (0.5 mg/kg; $p = 0.008$) or eticlopride (0.1 and 0.5 mg/kg; $p = 0.001$ and $p < 0.001$, respectively), compared to saline controls.

3.2.2 | Nociceptive responses in late phase chronic pain

Mechanical allodynia also increased post-surgery in CCI, but not in sham, rats that were tested for a CPP during a last phase of chronic pain. These effects were verified by a mixed-model repeated measures ANOVA, which revealed a significant surgery × time interaction in both morphine plus SCH23390, $F(20, 212) = 14.905$, $n = 59$, $p < 0.001$ (Figure 4c) and morphine plus eticlopride, $F(20, 220) = 23.147$, $n = 61$, $p < 0.001$ (Figure 4d) groups. There was no evidence that SCH23390 altered mechanical sensory responses in CCI rats (i.e., post hoc comparisons of CCI saline and SCH23390 groups, 0.1 mg/kg: $p = 0.985$; 0.5 mg/kg: $p = 0.997$), whereas CCI rats treated with eticlopride (0.1 mg/kg) showed lower responses than saline-treated rats on day 14 ($p = 0.004$). In both early and late phases of chronic pain, normality was violated in sham-lesioned groups receiving saline, $W = 0.552$, $p < 0.001$, reflecting the fact that some animals in these groups, as expected, displayed no withdrawal response to the filaments.

3.3 | Effects of DA D1 antagonism on place conditioning

Figure 5 shows that DA D1 antagonism had no motivational effects on its own. Neither CCI, $t(14) = -1.334$, $p = 0.203$, nor sham, $t(14) = -1.565$, $p = 0.140$, rats developed a preference for, or an aversion to, a compartment previously paired with SCH23390 (0.5 mg/kg).

3.4 | Effects of DA D1 antagonism on morphine-induced antinociception

Data presented in Figure 6, analyzed with a mixed-model repeated measures ANOVA, confirmed that DA D1 antagonism had no effect

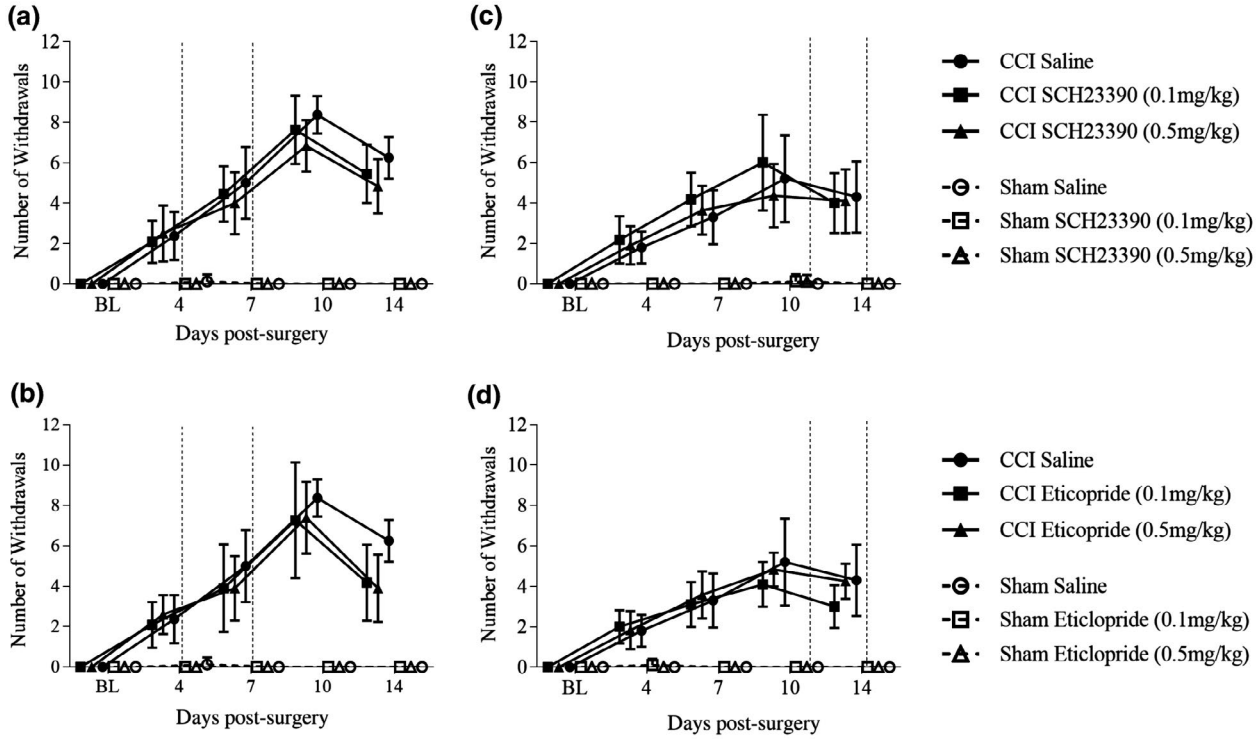


FIGURE 4 Nociceptive responses of chronic constriction injury (CCI) and sham-lesioned rats tested for a morphine conditioned place preference (CPP) during early (a, b) and late (c, d) phases of chronic pain. Data points represent mean (±*SD*) number of paw withdrawals (/10) to application of a von Frey filament in a test of mechanical allodynia. Dotted lines indicate CPP conditioning sessions during early and late phases of chronic pain in which animals were injected daily with morphine (2 or 6 mg/kg) combined with the dopamine (DA) D1 antagonist, SCH233390 (a, c), or the DA D2 antagonist, eticlopride (b, d). Baseline (BL) testing occurred 1 day prior to surgery and nociceptive testing was conducted in a drug-free state

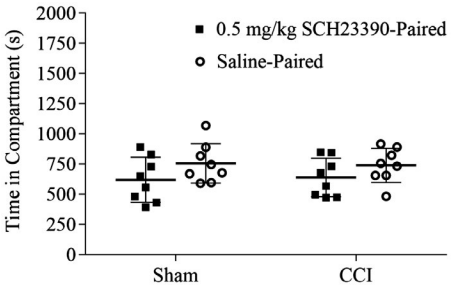


FIGURE 5 Effects of dopamine D1 antagonism on place conditioning in a late phase of chronic pain (11–15 days post-surgery). Bars represent mean (±*SD*) time spent in SCH23390- and saline-paired compartments over a 30-min drug-free test. Separate groups of chronic constriction injury (CCI) and sham-lesioned rats were injected with SCH233390 (0.5 mg/kg) or saline, administered 10 min prior to conditioning sessions

on the antinociceptive properties of morphine in either CCI or sham-lesioned rats. The ANOVAs revealed significant time x treatment interactions for withdrawal scores in early, $F(12, 80) = 6.091$, $n = 24$, $p < 0.001$ (Figure 6a), and late, $F(12, 80) = 9.721$, $n = 24$, $p < 0.001$ (Figure 6c), phases of chronic pain, with similar results in the tail flick test: $F(12, 80) = 7.416$, $n = 24$, $p < 0.001$ and $F(12, 80) = 10.320$, $n = 24$, $p < 0.001$ (Figure 6b,d). These patterns reflect increased nociceptive responses of CCI compared to sham-lesioned rats and a decline in the effectiveness of morphine across sessions (i.e., tolerance). Tukey's post hoc tests confirmed no significant differences between morphine plus saline versus morphine plus SCH 23390 groups on any measure (final test day of early and late phase chronic pain: withdrawal scores, $p = 0.937$ and $p = 0.971$, and tail flick latencies, $p = 0.794$ and $p = 0.984$).

4 | DISCUSSION

A primary finding in our study is that blockade of DA D1 receptors disrupts a morphine CPP in sham-lesioned, but not CCI, rats. The effect cannot be attributed to disruptions in memory processes as neuropathic rats treated with a D1 antagonist displayed a CPP. Nor did the drug produce motivational effects on its own, suggesting that the impact is specific to morphine reward in pain-naïve states. These findings parallel evidence that DA D1 antagonism reduces morphine reward in opiate-naïve, but not opiate-dependent, rats (Lintas et al., 2011). In contrast, D2 receptor blockade did not disrupt a morphine CPP in neuropathic rats, suggesting that alterations in reward processing in drug-dependent states (Lintas et al., 2011) and chronic pain may not overlap. Notably, opiate treatment in our experiment did not elicit any sign of withdrawal and was dramatically lower than the protocols used to establish morphine dependence in other CPP studies (Laviolette,

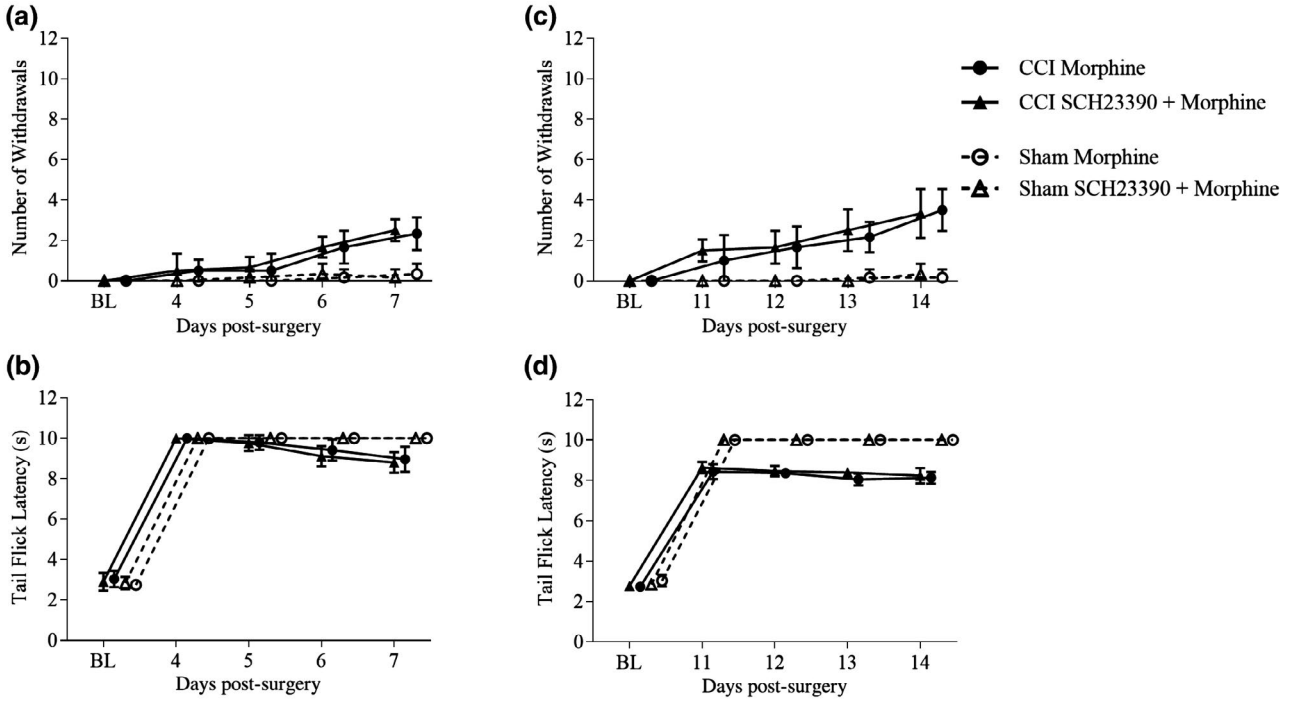


FIGURE 6 Effects of dopamine antagonism on morphine-induced anti-nociception during early (a, b) and late (c, d) phases of chronic pain. Data points represent mean (±*SD*) number of paw withdrawals (/10) to application of a von Frey filament (a, c) and tail flick latencies (b, d) in a test of thermal hyperalgesia. Separate groups of chronic constriction injury (CCI) and sham-lesioned rats were injected with morphine (2 or 6 mg/kg) combined with saline or SCH233390 (0.5 mg/kg) prior to nociceptive testing. Testing was conducted on days 4–7 or 11–14 post-surgery to coincide with conditioned place preference conditioning sessions in early and late phases of chronic pain, respectively. Baseline (BL) testing occurred 1 day prior to surgery

Nader, & Van der Kooy, 2002). Thus, pharmacological systems of morphine reward appear to be distinct in states of drug dependence and chronic pain, at least as measured in the CPP paradigm.

The fact that DA D1 antagonism had no effect on a morphine CPP in CCI rats strengthens the contention that neuropathic pain alters DAergic mechanisms of opiate reward (Asaoka et al., 2018; Taylor et al., 2016). This pain-induced shift in neuropharmacological systems of drug reward likely involves the alterations in DA signaling in the VTA to NAc pathway (Taylor et al., 2015). Subsequent studies have shown that chronic neuropathic pain decreases basal DA release within the NAc, which is positively correlated with a decrease in VTA dopaminergic cell firing (Ren, Centeno, & Berger, 2016). Importantly, this latter study suggested that DA hypofunction was at least partially responsible for pain hypersensitivities in neuropathic pain as treatment with levodopa attenuated mechanical tactile allodynia. Additionally, optogenetic activation of DA neurons attenuated thermal hyperalgesia in a model of neuropathic pain (Watanabe, Narita, & Hamada, 2018), demonstrating the importance of DA circuitry in chronic pain states. In contrast, optogenetic inhibition of VTA-NAc DA projection neurons reversed thermal hyperalgesia in a neuropathic pain model (Zhang, Qian, & Li, 2017). It is unclear why these latter studies report opposite findings, although the differences in outcomes may be due to the topographical organization of heterogeneous DA neurons, where cells in the dorsal and ventral VTA are inhibited or excited by noxious electrical stimuli, respectively (Matsumoto & Hikosaka, 2009). It is also possible that D1 antagonism is having an effect at other neural sites, such as the central amygdala (Zarrindast, Rezayof, Sahraei, Haeri-Rohani, & Rassouli, 2003) or hippocampus (Rezayof, Zarrindast, Sahraei, & Haeri-Rohani, 2003). Regardless, the inability of the D1 antagonist to block morphine reward in the CCI neuropathic pain model in our study supports the hypothesis that morphine is producing a place preference via negative reinforcement (rather than positive motivational valence) by alleviating activation of nociceptive circuitry.

Behavioral studies examining pain-induced alterations in reward processing have also produced conflicting results. Some evidence points to increased reward in neuropathic pain; a morphine CPP is expressed at lower doses in CCI, compared to pain-naïve, rats (Cahill et al., 2013; Woller et al., 2012) and inflammatory pain increases the motivation to self-administer high doses of heroin (Hipolito, Wilson-Poe, & Campos-Jurado, 2015). On the other hand, the rewarding effect of morphine is reduced in intracranial self-stimulation (Ewan & Martin, 2011) and intravenous self-administration (Martin, Kim, Buechler, Porreca, & Eisenach, 2007) when animals are experiencing chronic pain. The issue is difficult to untangle in paradigms that do not provide independent measures of positive and negative reinforcement (i.e., drug reward vs. relief from aversive states of pain). We recently used a one-sided CPP paradigm (Bechara et al., 1992; Bechara & Kooy, 1992) to dissociate these two effects, revealing that the negative affective states associated with chronic pain are

alleviated through a mechanism involving kappa-opioid receptors (Liu et al., 2019).

As expected, mechanical allodynia intensified following CCI surgery, manifested as increased withdrawal responses in the ipsilateral paw across testing. Nociceptive responses were lower on the last test day in CCI rats with a prior history of SCH 23390 treatment, but only in animals tested for a CPP in an early phase of chronic pain. It is unlikely that this change impacted measures of morphine reward in that CPP conditioning and testing were completed 1 week prior to the last nociceptive test. Repeated administration of a D2 receptor antagonist reduced final day pain scores in both experiments, but this manipulation had no effect on a morphine CPP. More importantly, subsequent testing confirmed that our drug regime which reduced a morphine CPP (i.e., high dose SCH 23390) did not impact morphine-induced analgesia in either chronic pain or pain-naïve states. It is unlikely, therefore, that D1 receptor blockade disrupted reward by altering nociceptive responses during CPP conditioning sessions. This is consistent with a lack of evidence for D1 receptor antagonists altering morphine-induced analgesia in chronic pain, although these drugs may attenuate the antinociceptive effect of opiates in measures of acute (Flores, El Banoua, Galán-Rodríguez, & Fernandez-Espejo, 2004) or tonic (Altier & Stewart, 1999) pain.

We cannot rule out the possibility that DA receptor blockade would disrupt morphine reward at later time points post-surgery, as functional changes in DA responses to rewarding stimuli continue to evolve up to 30 days following nerve ligation (Kato et al., 2016). We selected a shorter time window for behavioral assessment because nociceptive reflex responses in our CCI model peak by day 10. It is also possible that D1 receptor antagonism altered locomotor responses to morphine during CPP conditioning sessions, which then impacted the expression of a morphine CPP. This seems unlikely given that central infusions of a D1 receptor antagonist block morphine reward, while having no effect on morphine-induced locomotion (Zarrindast et al., 2003). A similar dissociation is observed in mice lacking D1 receptors (Urs, Daigle, & Caron, 2011), although these effects are not consistent across studies (Wang et al., 2015). Indeed, there is debate as to whether the genetic deletion of D1 receptors blocks a morphine CPP (Urs et al., 2011; Wang et al., 2015), possibly reflecting differences in developmental adaptations that impact receptor function in adulthood.

In conclusion, our study adds to evidence for pain-induced alterations in reward processing and suggests that changes in DA D1 receptor function may contribute to this effect. Identifying mechanisms that underlie shifts in drug reward following nerve injury may help to alleviate the devastating impacts of chronic pain, which include reduced quality of life (Doth, Hansson, Jensen, & Taylor, 2010; Langley, Van Litsenburg, Cappelleri, & Carroll, 2013) and exacerbation of comorbid disorders, such as anxiety and depression (DosSantos, Moura, & DaSilva, 2017). Few patients suffering from neuropathic pain receive adequate treatment (Torrance, Ferguson, & Afolabi, 2013) and current pharmaceutical therapies show limited efficacy and large placebo responses for modest outcomes (Finnerup, Attal, & Haroutounian, 2015). Developing compounds that target altered reward processing may be a more fruitful direction for treating this condition.

ACKNOWLEDGMENTS

We thank Meghan Luhowy and Lihua Xue for data collection, Lisa Willberforce and Roland Dupras for technical assistance, and Amanda Maracle for statistical advice. Queen's University is situated on traditional Anishinaabe and Haudenosaunee Territory.

CONFLICT OF INTEREST

No conflicting interests exist.

AUTHOR CONTRIBUTIONS

All authors read and approved the manuscript. *Conceptualization*, C.M.C. and M.C.O.; *Methodology*, P.G., M.C.M., and M.C.O.; *Investigation*, P.G. and C.M.C.; *Formal Analysis*, M.C.O.; *Data Curation*, P.G. and M.C.M.; *Writing – Original Draft*, P.G. and M.C.O.; *Writing – Review & Editing*, P.G., M.C.M., C.M.C., and M.C.O.; *Funding Acquisition*, C.M.C. and M.C.O.

ORCID

Mary C. Olmstead https://orcid.org/0000-0003-2249-4411

DATA AVAILABILITY STATEMENT

Data are available through Scholars Portal Dataverse, an institutional repository at Queen's University. Data can be accessed using the following https://doi.org/10.5683/SP2/CTTENF.

REFERENCES

Altier, N., & Stewart, J. (1999). The role of dopamine in the nucleus accumbens in analgesia. *Life Sciences*, *65*, 2269–2287. https://doi.org/10.1016/S0024-3205(99)00298-2

Asaoka, Y., Kato, T., Ide, S., Amano, T., & Minami, S. (2018). Pregabalin induces conditioned place preference in the rat during the early, but not late, stage of neuropathic pain. *Neuroscience Letters*, *668*, 133–137. https://doi.org/10.1016/j.neulet.2018.01.029

Bechara, A., Harrington, F., Nader, K., & van der Kooy, D. (1992). Neurobiology of motivation: Double dissociation of two motivational mechanisms mediating opiate reward in drug-naive versus drug-dependent animals. *Behavioral Neuroscience*, *106*, 798–807. https://doi.org/10.1037/0735-7044.106.5.798

Bechara, A., & van der Kooy, D. (1992). A single brain stem substrate mediates the motivational effects of both opiates and food in nondeprived but not deprived states. *Behavioral Neuroscience*, *106*, 351–363.

Bennett, G. J., & Xie, Y. K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*, *33*, 87–107. https://doi.org/10.1016/0304-3959(88)90209-6

Berkotitz, B., Cerreta, K., & Spector, K. (1974). The influence of physiologic and pharmacologic factors on the disposition of morphine as determined by radioimmunoassay. *Journal of Pharmacology and Experimental Therapeutics*, *191*, 527–534.

Blyth, F. M., March, L. M., Brnabic, A. J. M., Jorm, L. R., Williamson, M., & Cousins, M. J. (2001). Chronic pain in Australia: A prevalence study. *Pain*, *89*, 127–134. https://doi.org/10.1016/S0304-3959(00)00355-9

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain*, *10*, 287–333. https://doi.org/10.1016/j.ejpain.2005.06.009

Cahill, C. M., Xue, L., Grenier, P., Magnussen, C., Lecour, S., & Olmstead, M. C. (2013). Changes in morphine reward in a model of neuropathic pain. *Behavioural Pharmacology*, *24*, 207–213. https://doi.org/10.1097/FBP.0b013e3283618ac8

Christie, M. J. (2008). Cellular neuroadaptations to chronic opioids: Tolerance, withdrawal and addiction. *British Journal of Pharmacology*, *154*, 384–396. https://doi.org/10.1038/bjp.2008.100

Contet, C., Kieffer, B. L., & Befort, K. (2004). Mu opioid receptor: A gateway to drug addiction. *Current Opinion in Neurobiology*, *14*, 370–378. https://doi.org/10.1016/j.conb.2004.05.005

DosSantos, M. F., Moura, B. d. S., & DaSilva, A. F. (2017). Reward circuitry plasticity in pain perception and modulation. *Frontiers in Pharmacology*, *8*, 790. https://doi.org/10.3389/fphar.2017.00790

Doth, A. H., Hansson, P. T., Jensen, M. P., & Taylor, R. S. (2010). The burden of neuropathic pain: A systematic review and meta-analysis of health utilities. *Pain*, *149*, 338–344. https://doi.org/10.1016/j.pain.2010.02.034

Evans, C. J., & Cahill, C. M. (2016). Neurobiology of opioid dependence in creating addiction vulnerability. *F1000Research*, *5*, 1748.

Ewan, E. E., & Martin, T. H. (2011). Opioid facilitation of rewarding electrical stimulation is suppressed in rats with neuropathic pain. *Anesthesiology*, *114*, 624–632.

Finnerup, N. B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R. H., … Wallace, M. (2015). Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. *The Lancet Neurology*, *14*, 162–173. https://doi.org/10.1016/S1474-4422(14)70251-0

Flores, J. A., El Banoua, F., Galán-Rodríguez, B., & Fernandez-Espejo, E. (2004). Opiate anti-nociception is attenuated following lesion of large dopamine neurons of the periaqueductal grey: Critical role for D1(not D2) dopamine receptors. *Pain*, *110*, 205–214. https://doi.org/10.1016/j.pain.2004.03.036

Glass, G. V., Peckham, P. D., & Sanders, J. R. (1972). Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *Review of Educational Research*, *42*, 237–288.

Hagelberg, N., Forssell, H., Aalto, S., Rinne, J. O., Scheinin, H., Taiminen, T., … Jääskeläinen, S. K. (2003). Altered dopamine D2 receptor binding in atypical facial pain. *Pain*, *106*, 43–48. https://doi.org/10.1016/S0304-3959(03)00275-6

Hagelberg, N., Forssell, H., Rinne, J. O., Scheinin, H., Taiminen, T., Aalto, S., … Jääskeläinen, S. (2003). Striatal dopamine D1 and D2 receptors in burning mouth syndrome. *Pain*, *101*, 149–154. https://doi.org/10.1016/S0304-3959(02)00323-8

Hipólito, L., Wilson-Poe, A., Campos-Jurado, Y., Zhong, E., Gonzalez-Romero, J., Virag, L., … Bruchas, M. R. (2015). Inflammatory pain promotes increased opioid self-administration: Role of dysregulated ventral tegmental area opioid receptors. *Journal of Neuroscience*, *35*, 12217–12231.

Johannes, C. B., Le, T. K., Zhou, X., Johnston, J. A., & Dworkin, R. H. (2010). The prevalence of chronic pain in United States adults: Results of an internet-based survey. *Journal of Pain*, *11*, 1230–1239. https://doi.org/10.1016/j.jpain.2010.07.002

Kato, T., Ide, S., & Minami, M. (2016). Pain relief induces dopamine release in the rat nucleus accumbens during the early but not late phase of neuropathic pain. *Neuroscience Letters*, *629*, 73–78. https://doi.org/10.1016/j.neulet.2016.06.060

Kleinschnitz, C., Brinkhoff, J., Zelenka, M., Sommer, C., & Stoll, G. (2004). The extent of cytokine induction in peripheral nerve lesions depends on the mode of injury and NMDA receptor signaling. *Journal of Neuroimmunology*, *149*, 77–83. https://doi.org/10.1016/j.jneuroim.2003.12.013

Langley, P. C., Van Litsenburg, C., Cappelleri, J. C., & Carroll, D. (2013). The burden associated with neuropathic pain in Western Europe. *Journal of Medical Economics*, *16*, 85–95. https://doi.org/10.3111/13696998.2012.729548

Laviolette, S. R., Nader, K., & Van der Kooy, D. (2002). Motivational state determines the functional role of the mesolimbic dopamine system in the mediation of opiate reward processes. *Behavioral Brain Research*, *129*, 17–29. https://doi.org/10.1016/S0166-4328(01)00327-8

Lintas, A., Chi, N., Lauzon, N. M., Bishop, S. F., Gholizadeh, S., Sun, N., … Laviolette, S. R. (2011). Identification of a dopamine receptor-mediated opiate reward memory switch in the basolateral amygdala-nucleus accumbens circuit. *Journal of Neuroscience*, *31*, 11172–11183. https://doi.org/10.1523/JNEUROSCI.1781-11.2011

Liu, S. S., Pickens, S., Burma, N. E., Ibarra-Lecue, I., Yang, H., Xue, L., … Komarek, K. (2019). Kappa opioid receptors drive the tonic aversive component of chronic pain. *Journal of Neuroscience*, *39*, 4162–4178.

Mao, J. (2002). Opioid-induced abnormal pain sensitivity: Implications in clinical opioid therapy. *Pain*, *100*, 213–217. https://doi.org/10.1016/S0304-3959(02)00422-0

Martin, T. H., Kim, S. A., Buechler, N. L., Porreca, F., & Eisenach, J. C. (2007). Opioid self-administration in the nerve-injured rat: Relevance of antiallodynic effects to drug consumption and effects of intrathecal analgesics. *Anesthesiology*, *106*, 312–322. https://doi.org/10.1097/00000542-200702000-00020

Matsumoto, M., & Hikosaka, O. (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature*, *459*, 837–841. https://doi.org/10.1038/nature08028

Ozaki, S., Narita, M., Narita, M., Iino, M., Sugita, J., Matsumura, Y., & Suzuki, T. (2002). Suppression of the morphine-induced rewarding effect in the rat with neuropathic pain: Implication of the reduction in μ-opioid receptor functions in the ventral tegmental area. *Journal of Neurochemistry*, *82*, 1192–1198.

Ren, W., Centeno, M. V., Berger, S., Wu, Y., Na, X., Liu, X., … Surmeier, D. J. (2016). The indirect pathway of the nucleus accumbens shell amplifies neuropathic pain. *Nature Neuroscience*, *19*, 220–222. https://doi.org/10.1038/nn.4199

Rezayof, A., Zarrindast, M. R., Sahraei, H., & Haeri-Rohani, A. (2003). Involvement of dopamine receptors on the acquisition and expression of morphine-induced place preference in rats. *Journal of Psychopharmacology*, *17*, 415–423.

Schopflocher, D., Taenzer, P., & Jovey, R. (2011). The prevalence of chronic pain in Canada. *Pain Research and Management*, *16*, 445–450. https://doi.org/10.1155/2011/876306

Schulteis, G., Markou, A., Gold, L. H., Stinus, L., & Koob, G. F. (1994). Relative sensitivity to naloxone of multiple indices of opiate withdrawal: A quantitative dose-response analysis. *Journal of Pharmacology and Experimental Therapeutics*, *271*, 1391–1398.

Taylor, A. M., Becker, S., Schweinhardt, P., & Cahill, C. M. (2016). Mesolimbic dopamine signaling in acute and chronic pain: Implications for motivation, analgesia, and addiction. *Pain*, *157*, 1194–1198. https://doi.org/10.1097/j.pain.0000000000000494

Taylor, A. M. W., Castonguay, A., Taylor, A. J., Murphy, N. P., Ghogha, A., Cook, C., … Cahill, C. M. (2015). Microglia disrupt mesolimbic reward circuitry in chronic pain. *Journal of Neuroscience*, *35*, 8442–8450. https://doi.org/10.1523/JNEUROSCI.4036-14.2015

Torrance, N., Ferguson, J. A., Afolabi, E., Bennett, M. I., Serpell, M. G., Dunn, K. M., & Smith, B. H. (2013). Neuropathic pain in the community: More under-treated than refractory? *Pain*, *154*, 690–699. https://doi.org/10.1016/j.pain.2012.12.022

Trang, T., Al-Hasani, R., Salvemini, D., Salter, M. W., Gutstein, H., & Cahill, C. M. (2015). Pain and poppies: The good, the bad, and the ugly of

opioid analgesics. *Journal of Neuroscience*, *35*, 13879–13888. https://doi.org/10.1523/JNEUROSCI.2711-15.2015

Urs, N. M., Daigle, T. L., & Caron, M. G. (2011). A dopamine D1 receptor-dependent B-arrestin signaling complex potentially regulates morphine-induced psychomotor activation but not reward in mice. *Neuropsychopharmacology*, *36*, 561–568. https://doi.org/10.1038/npp.2010.186

Volkow, N. D., Koob, G. F., & McLellan, A. T. (2016). Neurobiologic advances from the brain disease model of addiction. *New England Journal of Medicine*, *374*, 363–371. https://doi.org/10.1056/NEJMra1511480

Volkow, N. D., & McLellan, A. T. (2016). Opioid abuse in chronic pain—Misconceptions and mitigation strategies. *New England Journal of Medicine*, *374*, 1253–1263. https://doi.org/10.1056/NEJMra1507771

Wang, Y. P., Wei, S. G., Zhu, Y. S., Zhao, B., Xun, X., & Lai, J. H. (2015). Dopamine receptor D1 but not D3 essential for morphine-induced conditioned responses. *Genetics and Molecular Research*, *14*, 180–189. https://doi.org/10.4238/2015.January.16.1

Watanabe, M., Narita, M., Hamada, Y., Yamashita, A., Tamura, H., Ikegami, D., … Narita, M. (2018). Activation of ventral tegmental area dopaminergic neurons reverses pathological allodynia resulting from nerve injury or bone cancer. *Molecular Pain*, *14*, 1–11. https://doi.org/10.1177/1744806918756406

Wise, R. A. (2008). Dopamine and reward: The anhedonia hypothesis 30 years on. *Neurotoxicity Research*, *14*, 169–183. https://doi.org/10.1007/BF03033808

Woller, S. A., Moreno, G. L., Hart, N., Wellman, P. J., Grau, J. W., & Hook, M. A. (2012). Analgesia or addiction?: Implications for morphine use after spinal cord injury. *Journal of Neurotrauma*, *29*, 1650–1662. https://doi.org/10.1089/neu.2011.2100

Wood, P. B., Schweinhardt, P., Jaeger, E., Dagher, A., Hakyemez, H., Rabiner, E. A., … Chizh, B. A. (2007). Fibromyalgia patients show an abnormal dopamine response to pain. *European Journal of Neuroscience*, *25*, 3576–3582. https://doi.org/10.1111/j.1460-9568.2007.05623.x

Zarrindast, M.-R., Rezayof, A., Sahraei, H., Haeri-Rohani, A., & Rassouli, Y. (2003). Involvement of dopamine D1 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. *Brain Research*, *965*, 212–221. https://doi.org/10.1016/S0006-8993(02)04201-4

Zhang, H., Qian, Y.-L., Li, C., Liu, D., Wang, L., Wang, X.-Y., … Cao, J.-L. (2017). Brain-derived neurotrophic factor in the mesolimbic reward circuitry mediates nociception in chronic neuropathic pain. *Biological Psychiatry*, *82*, 608–618. https://doi.org/10.1016/j.biopsych.2017.02.1180

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Transparent Peer Review Report

How to cite this article: Grenier P, Mailhiot MC, Cahill CM, Olmstead MC. Blockade of dopamine D1 receptors in male rats disrupts morphine reward in pain naïve but not in chronic pain states. *J Neuro Res*. 2019;00:1–12. https://doi.org/10.1002/jnr.24553