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## Evaluation of reward from pain relief

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### Abstract

The human experience of pain is multidimensional and comprises sensory, affective, and cognitive dimensions. Preclinical assessment of pain has been largely focused on the sensory features that contribute to nociception. The affective (aversive) qualities of pain are clinically significant but have received relatively less mechanistic investigation in preclinical models. Recently, operant behaviors such as conditioned place preference, avoidance, escape from noxious stimulus, and analgesic drug self-administration have been used in rodents to evaluate affective aspects of pain. An important advance of such operant behaviors is that these approaches may allow the detection and mechanistic investigation of spontaneous neuropathic or ongoing inflammatory/nociceptive (i.e., nonevoked) pain that is otherwise difficult to assess in nonverbal animals. Operant measures may allow the identification of mechanisms that contribute differentially to reflexive hypersensitivity or to pain affect and may inform the decision to progress novel mechanisms to clinical trials for pain therapy. Additionally, operant behaviors may allow investigation of the poorly understood mechanisms and neural circuits underlying motivational aspects of pain and the reward of pain relief.

### Keywords

operant behavior; conditioned place preference; spontaneous pain; pain affect; dopamine; mesolimbic reward circuit

### Introduction

Pain is part of the body's defense system and serves as a protective mechanism for the organism's survival. Pain facilitates fast withdrawal from a damaging situation (reflexive reaction) and safe-guarding of the affected body part while it heals, and provides a strong teaching signal that enables learning to avoid similar harmful situations in the future.<sup>1</sup> Pain

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#### Conflicts of interest

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is a complex multidimensional subjective experience comprising sensory/discriminative, affective/motivational, and cognitive/evaluative components.

While the affective aspects of pain are the most bothersome to patients,<sup>2,3</sup> the focus of preclinical research has largely been on the neurobiology of somatosensation (nociception) that can elicit sensations of pain. It is unlikely to be possible to directly measure pain affect in an animal. As a consequence, very little is known about the molecular mechanisms and neural circuitry that underlie the affective components of spontaneous neuropathic or ongoing inflammatory/nociceptive pain. Basic pain-motivated behavior is fundamental to survival and appears to be encoded by phylogenetically ancient neural circuits that are conserved between humans and rodents.<sup>4,5</sup> Recent preclinical research has attempted to capture the aversive aspects of pain, using the motivational drive of animals to avoid pain and seek relief (safety) from ongoing or spontaneous pain. Motivation to maintain a normal (non-painful) state facilitates learning to avoid behaviors that result in harm or injury. In operant conditioning, these principles form the basis of negative reinforcement and can be used to indirectly assess the affective aspects of pain in laboratory animals.

Operant learning behaviors such as conditioned place preference (CPP)/avoidance,<sup>6</sup> escape from a noxious stimulus,<sup>7</sup> and systemic and spinal self-administration of analgesic drugs<sup>8</sup> have begun to be used to study motivational aspects of pain. Unlike reflex responses that can be induced in decerebrate animals,<sup>9,10</sup> operant behaviors require cerebral processing and learning. Operant measures may therefore allow the evaluation and study of the supraspinal pain circuits that underlie the conscious awareness of pain. As discussed below, operant behaviors may reveal the presence of ongoing or spontaneous (i.e., nonevoked) pain, which is otherwise difficult to assess in nonverbal animals. Operant measures also permit evaluation of new drugs to elicit relief of spontaneous neuropathic or ongoing inflammatory pain. Importantly, mechanisms that mediate such nonevoked pain may differ from those promoting nociceptive hypersensitivity reflected in enhanced reflexive withdrawal responses (hyperreflexia).<sup>11</sup> Additionally, operant behaviors allow investigations of the mechanisms and brain neural circuits underlying the reward of pain relief. Because motivated behavior and the neural circuits mediating aversiveness and reward are highly conserved across species,<sup>4</sup> information from operant behavior studies will likely be of high translational relevance to the discovery of therapies for human pain. However, the limitations of animal models in studying the complex human pain experience must be acknowledged.

Most operant measures of pain use approaches in which the animal learns to escape from a painful situation or to perform a behavior that terminates pain (i.e., drug self-administration or self-stimulation). The place escape/avoidance paradigm evaluates the level of aversiveness to an evoked nociceptive stimulation by measuring the latencies and duration of escape from an area where noxious stimulation occurs.<sup>7,12</sup> For example, aversion to mechanical nociceptive stimulation was evaluated using a place escape/avoidance paradigm in animals with unilateral hind paw inflammatory or neuropathic pain.<sup>7,13,14</sup> In this paradigm, animals must make a choice between the dark (preferred) side of the chamber, where they receive repeated mechanical stimulation with a von Frey filament to the ipsilateral (i.e., injured) paw, and the light (nonpreferred) side of the chamber where they receive stimulation to the contralateral paw. The amount of time the animal spends in the

light side of the chamber is a measure of aversion to mechanically evoked pain. To characterize orofacial pain, an operant behavior assay has been developed in which an animal must press its face against a stimulus thermode to receive a drink reward. This presents a conflict between positive reward and noxious thermal stimulation. The number and duration of facial contacts has been suggested to reflect the intensity of orofacial pain.<sup>15</sup> Aversion to noxious heat or cold stimulation can be assessed using an area with thermally regulated floor surface and a normally nonpreferred (i.e., illuminated) escape platform.<sup>12</sup> Another indirect measure of pain in animals is intrathecal or systemic self-administration of an analgesic drug<sup>8</sup> or depression of intracranial self-stimulation.<sup>16</sup> Finally, conditioned place preference/avoidance testing, described in detail here, can reveal the presence of ongoing or spontaneous (i.e., nonevoked) pain and pain relief based on an animal's preference for the context paired with a pain-relieving treatment. There are potential confounds in all operational assays that need to be considered: (1) sensitivity to attention, learning, and motor impairments, and (2) sensitivity to intrinsic rewarding/motivational properties of the drug treatments.

## **Use of conditioned place preference to capture aversiveness of ongoing or spontaneous pain**

### **Conditioned place preference procedure**

Conditioned place preference (CPP) can be used to reveal the presence of pain and to help in validation of mechanisms that can elicit relief. The procedure can be performed with either multitrial or single trial conditioning protocols as previously described.<sup>6,17</sup> CPP boxes often consist of two conditioning chambers distinguished by visual and sensory cues that are connected by a middle (neutral) chamber. Rats undergo handling by the experimenter prior to the preconditioning phase. A single trial protocol consists of one preconditioning, one conditioning, and one test day. On the preconditioning day, rats are placed into the CPP boxes with access to all chambers. Time spent in each chamber is recorded across 15 min without the investigator in the room and subsequently analyzed to verify no preconditioning chamber preference. Animals spending more than 80% or less than 20% of total time in one chamber are eliminated from the study. On the conditioning day, rats receive vehicle treatment and are then immediately (within 2 min) placed in the appropriate pairing chamber for 30 min with no access to other chambers. Four hours later, rats receive drug treatment and are then immediately confined within the opposite pairing chamber for 30 min. Chamber pairings are counterbalanced. On the test day, 20 h following the afternoon pairing, rats are placed in the CPP box with access to all chambers and time spent within each chamber is recorded for 15 min and analyzed for chamber preference. Of note, on the test day, animals are drug-free when placed in the chambers; therefore any possible motor/sedative effects of the drug do not impact the time spent in each chamber. Increased time spent in the drug-paired chamber and corresponding decreased time spent in the alternate chambers indicates preference for the drug or treatment.

### **Conditioned place preference reveals the presence of ongoing pain**

Injury typically results in hypersensitivity to evoked stimuli and ongoing pain that may be time dependent or long lasting. Evoked mechanical or thermal hypersensitivity can be

reliably measured in laboratory animals, but the presence of ongoing pain is more difficult to demonstrate in nonverbal animals. Pain aversiveness provides strong motivation to seek pain relief. Thus, pairing treatments that produce pain relief with a context will produce CPP, revealing the presence of ongoing inflammatory or spontaneous neuropathic pain. Such pain is referred to as nonevoked, and much debate has ensued regarding appropriate terminology and whether evoked stimuli could represent physiological processes associated with daily life activities (e.g., local systolic/diastolic changes in diameter of blood vessels that lie near nerves).<sup>18</sup> In this review, we use the term evoked to refer to a stimulus that is applied by the experimenter (e.g., a normally innocuous von Frey filament or noxious thermal stimulus) and nonevoked to refer to pain that is simply present (i.e., spontaneous or ongoing). We have used experimental pain models that may elicit time-related spontaneous neuropathic or ongoing inflammatory/nociceptive pain, and determined CPP in response to clinically used pain-relieving treatments that have rapid onset of effect (Table 1).<sup>17,19-21</sup>

**Ongoing neuropathic pain**—Following experimental neuropathic pain produced by ligation of the L5 and L6 spinal nerves (SNL),<sup>25</sup> the rats display evoked thermal and tactile hypersensitivity. In injured rats, intrathecal administration of either an  $\alpha_2$  adrenergic receptor agonist clonidine or an N-type calcium channel blocker  $\omega$ -conotoxin alleviated evoked hypersensitivity and produced preference for the chamber paired with the treatment.<sup>6</sup> Of critical importance, the same treatments did not elicit CPP in sham-operated control rats, confirming that these treatments are nonrewarding in the absence of pain. Thus, without evoked stimulation, SNL rats experience spontaneous neuropathic pain that is revealed by CPP. Additionally, these results demonstrate that clonidine or  $\omega$ -conotoxin, drug treatments that have been proven to alleviate pain in humans,<sup>26,27</sup> relieve both evoked hypersensitivity and spontaneous, nonevoked neuropathic pain. Neuropathic pain has been shown to induce neuroplasticity, including enhanced descending pain facilitation from the rostral ventromedial medulla (RVM).<sup>28</sup> Accordingly, inactivation of the RVM with microinjection of lidocaine relieves both tactile and thermal hypersensitivity.<sup>28</sup> Importantly, in neuropathic, but not control, rats RVM lidocaine also elicited CPP, indicating relief of spontaneous pain.<sup>6</sup> Therefore, intrinsically nonrewarding treatments may become rewarding if they provide relief of ongoing or spontaneous pain.

**Ongoing inflammatory pain**—A time-dependent rat model of inflammatory pain was used to demonstrate the temporal dissociation of evoked and ongoing pain.<sup>17</sup> Intraplantar complete Freund's adjuvant (CFA) elicited thermal hyperalgesia and guarding behavior that was observed during a one to four day period following CFA administration. Intrathecal clonidine administration blocked CFA-induced thermal hyperalgesia at both time points post-CFA. In contrast, spinal clonidine produced CPP in CFA-treated rats only one day, but not four days, following CFA injection. These results indicate that in time-dependent pain conditions, such as those involving acute inflammatory stimuli, evoked and ongoing pain have different time courses. Consequently, the mechanisms and treatment options for inflammatory evoked and ongoing pain may be different and need to be investigated using appropriate behavioral methods.

**Ongoing postsurgical pain**—Similar temporal dependence was also observed in a rat model of postsurgical pain. Incision of the skin and deep tissue in rat's hind paw<sup>29,30</sup> produced thermal hyperalgesia lasting for four days following the surgery. This hyperalgesia was reversed by peripheral nerve block (PNB) with lidocaine injection into the ipsilateral popliteal fossa. One day after the surgery, injured rats displayed prominent hind paw guarding behavior, which has been suggested to reflect the presence of spontaneous or ongoing pain similar to pain at rest in postoperative patients.<sup>30,31</sup> Guarding behavior was greatly diminished at four days postincision. Similarly, we have shown in a conditioning study that PNB elicited CPP only at one day but not four days following the injury.<sup>24</sup> The same time dependence was also observed for spontaneous activity of nociceptors in injured rats determined by *in vivo* single-fiber electrophysiological recordings.<sup>31</sup> Therefore, ongoing postsurgical pain reflected by guarding and CPP is likely to be driven by spontaneous nociceptor activity. This may explain why ongoing pain may resolve at an earlier time after injury, while evoked pain still persists. This conclusion is in agreement with clinical observations of human postsurgical pain associated with an initial period of strong, ongoing pain that transitions in a time-dependent manner to tenderness and hypersensitivity to evoked stimuli such as movement.<sup>32</sup> These behavioral data suggest that CPP reflects the removal of the aversive state associated with ongoing pain, but not hypersensitivity.

**Ongoing osteoarthritic pain**—Similarly, we investigated the presence of ongoing pain in a rat model of osteoarthritis (OA).<sup>20</sup> Injection of monosodium iodoacetate (MIA) into the intra-articular space of the knee is a well-established model of OA pain<sup>33-35</sup> that is characterized by dose- and time-dependent cartilage erosion and joint degeneration resulting in changes in weight bearing and hypersensitivity to tactile and thermal stimuli.<sup>36</sup> However, whether ongoing pain is present in this model is not clear. We treated rats with a high dose of MIA (4.8 mg) to model severe, NSAID-resistant OA. Twenty-eight days after the MIA treatment, CPP was demonstrated to intrathecal clonidine.<sup>20</sup> Similarly, peripheral nerve block with intra-articular administration of lidocaine, effective at relieving pain in OA patients,<sup>37</sup> elicited CPP in animals treated with a high dose of MIA (4.8 mg), but could not be demonstrated for rats treated with lower doses (1 or 3 mg).<sup>23</sup> Peripheral nerve block in the contralateral knee joint was completely ineffective, indicating that CPP in MIA-induced OA pain is elicited by local nerve blockade but not by systemic effects of lidocaine. All doses of MIA produced evoked hypersensitivity and weight bearing asymmetry. Treatment with diclofenac, an NSAID used to treat OA pain in patients,<sup>38</sup> effectively reversed weight bearing asymmetry at all MIA doses, but failed to block CPP from intra-articular lidocaine in the high-dose MIA group. These findings suggest that high-dose MIA may be more relevant to NSAID-resistant OA pain and that weight-bearing does not capture ongoing pain in the animal model. Thus, while joint malfunctions induced by lower doses of MIA are sufficient to produce behavioral changes apparently relevant to some aspects of OA pain, including hypersensitivity, these doses may not elicit a sufficiently severe aversive state and associated motivated behavior that can be detected with CPP. The presence of spontaneous pain at higher doses of MIA may reflect more advanced joint destruction that could be associated with nerve damage. The results may indicate potential dissociation of the mechanisms underlying evoked and spontaneous OA pain.

**CPP is not observed following treatments ineffective for ongoing pain**—Our studies have indicated that relief of ongoing or spontaneous pain, regardless of the injury model, elicits CPP. An important point is that the treatments, including peripheral nerve block or intrathecal administration of  $\omega$ -conotoxin<sup>26</sup> or clonidine,<sup>27,39</sup> did not produce CPP in uninjured animals, implying that the treatments themselves are not rewarding. Thus, drugs that do not elicit reward (and CPP) in the absence of injury might do so only in the presence of ongoing pain. Additionally, peripheral nerve blockade of the contralateral (uninjured) limb in rats with OA<sup>23</sup> or postsurgical pain<sup>24</sup> did not elicit CPP, confirming that it is pain relief that drives the behavior. It is also noted that treatments shown ineffective for ongoing pain in humans do not produce CPP in animal pain models. Spinal administration of adenosine blocks evoked hyperalgesia in patients with neuropathic pain, but fails to alleviate overall ongoing pain.<sup>40</sup> We used intrathecal administration of adenosine in the rat model of neuropathic pain and found that adenosine reversed tactile hypersensitivity but did not produce CPP.<sup>6</sup> Therefore, in agreement with clinical observations, spinal adenosine had no effect on spontaneous neuropathic pain at a dose at which it reverses tactile hypersensitivity. Manifestation of CPP in injured rats, together with the lack of CPP in uninjured rats, may be used to measure the effectiveness of the treatment to alleviate ongoing pain.

## Evaluating the effectiveness of new pain relief treatments and mechanisms

In the previous section, we described experiments that collectively suggested that ongoing or spontaneous pain can be revealed in multiple experimental neuropathic, inflammatory, and nociceptive models, with CPP resulting from clinically validated treatments. Additionally, we have also demonstrated that treatments that are not effective in relieving ongoing or spontaneous pain in humans do not elicit CPP in preclinical pain models. In these studies, CPP accurately reflected the effectiveness of clinical treatments, suggesting that CPP may be used to assess the potential effectiveness of new treatments and new mechanisms for relief of ongoing spontaneous pain.

An important consideration for the design of CPP experiments is the pharmacokinetic profile of pain-relieving treatments. To induce chamber preference, an animal must associate the treatment effects with the context of the CPP chamber. Therefore, only rapid onset treatments (e.g., onset within minutes), such as RVM lidocaine, intrathecal drug administration, or peripheral nerve block, can produce rapid pain relief and elicit CPP. Most systemically administered drugs, however, act more slowly, requiring more than 30 min to reach the full effect (note that chamber pairing is typically completed within 30 min following the treatment). However, the effects of slow-acting drugs can be revealed if they are given as a pretreatment that may produce relief of the pain-induced aversive state. Following systemic treatment, the animal can be challenged with a rapidly-acting pain reliever previously established to elicit CPP in the specified pain model. Thus, within the MIA model of osteoarthritis, we demonstrated that diclofenac pretreatment failed to block CPP to intra-articular lidocaine administered at the peak time point at which diclofenac normalized weight asymmetry, suggesting that the NSAID did not sufficiently alleviate advanced OA pain. Examples of the use of CPP testing to investigate the effectiveness of new drug treatments to relieve ongoing pain are summarized in Table 2 and discussed below.



### Role of TRPV1 receptors in ongoing pain

Transient receptor potential cation channel subfamily V member 1 (TRPV1; capsaicin receptor) receptors are currently under investigation as potential pharmacological targets for the treatment of inflammatory and neuropathic pain. The receptors are expressed primarily on small to medium-sized nociceptors and respond to noxious heat and acidic environments.<sup>41</sup> Tissue damage and inflammation may increase sensitivity of TRPV1 receptors to nociceptive signaling, resulting in thermal hyperalgesia and allodynia.<sup>42</sup> Whether TRPV1 promotes ongoing or spontaneous pain is not known. Resiniferatoxin (RTX), a potent TRPV1 agonist, produces long-lasting desensitization of TRPV1-expressing afferents, resulting in blockade of thermal, but not mechanical, hypersensitivity.<sup>43,44</sup> Using CPP, we have demonstrated that ongoing neuropathic (SNL)<sup>19</sup> and inflammatory (CFA)<sup>17</sup> pain were abolished by pretreatment of the animals with systemic RTX, indicating that ongoing inflammatory or neuropathic pain is likely driven by TRPV1-sensitive afferents from the site of injury. On the other hand, in rats with CFA-induced inflammatory pain, the selective TRPV1 antagonist AMG9810 had no effect on peripheral nerve block-induced CPP at a dose sufficient to block thermal hypersensitivity.<sup>17</sup> These results suggest that even though TRPV1 receptors contribute to inflammation-induced hyperalgesia, blockade of this receptor may not be sufficient to alleviate ongoing inflammatory pain. This further underscores differential mechanisms of evoked and ongoing pain.

### Role of rostral anterior cingulate cortex in ongoing pain

Neuroimaging studies of chronic pain in patients as well as acute pain in volunteers implicate activation of the rostral anterior cingulate cortex (rACC) in the aversive aspects of pain.<sup>45,46</sup> In animal studies, lesions of the rACC have been shown to abolish formalin-induced conditioned place aversion without altering evoked hypersensitivity.<sup>47</sup> A potential mechanism of pain aversiveness may involve protein kinase M $\zeta$  (PKM $\zeta$ )-mediated amplification of nociceptive processing in the rACC.<sup>48</sup> We have investigated the possible role of PKM $\zeta$ -mediated amplification in the rACC in ongoing neuropathic pain in rats with spinal nerve ligation.<sup>22</sup> In SNL rats, blockade of the descending pain facilitation by RVM lidocaine elicits CPP (Table 1). Bilateral lesions of the rACC abolished CPP without altering SNL-induced evoked pain. In addition, blockade of PKM $\zeta$  activity with  $\zeta$ -inhibitory pseudosubstrate peptide (ZIP), but not a scrambled ZIP peptide, also reversed nerve injury-induced spontaneous, but not evoked, pain.

From these examples, it is clear that the mechanisms of ongoing pain are different than the mechanisms of evoked pain. Importantly, pain experience is modulated at supraspinal circuits by other motivations, including stress and fear, and other factors such as attention and mood.<sup>49,50</sup> Evoked measures of pain do not require brain processing. Therefore, to study supraspinal mechanisms of pain, operant measures of pain such as CPP provide an approach that complements existing measures and offers different mechanistic information.

## CPP demonstrates the engagement of mesolimbic reward circuits in pain relief

The underlying neural mechanisms of positively reinforced behavior elicited by, for example, natural rewards of food or water involve activation of the brain's mesolimbic reward pathway.<sup>51,52</sup> Remarkably, rewarding drugs exert their effects by activating the same circuits.<sup>53</sup> The neural circuits that are associated with the rewards that result from relief of aversive states, including pain, have received much less attention.<sup>54</sup> We have used CPP to investigate the role of mesolimbic reward circuits in pain relief reward.<sup>24</sup>

### Mesolimbic dopamine system and positive reinforcement

The mesolimbic reward circuit comprising the ventral tegmental area (VTA) and its dopaminergic projections to the nucleus accumbens (NAc)<sup>55</sup> is stimulated by positive motivational states associated with natural rewards, rewarding drugs, and reward-predicting stimuli.<sup>51-53</sup> Manipulations that disrupt mesolimbic dopamine transmission attenuate food or drug reward-induced CPP.<sup>56,57</sup> Electrophysiological recordings from dopaminergic neurons in the VTA demonstrate phasic neuronal activation by primary food or liquid rewards, rewarding drugs, and reward-predicting cues.<sup>58</sup> Similarly, immunohistochemical studies show increased expression of the immediate early gene *cFos* in the VTA in response to rewarding drugs, providing further support for an enhanced neuronal activity.<sup>53,59-61</sup> The NAc can be anatomically and functionally divided into core and shell regions that respectively receive projections from the lateral and medial VTA.<sup>62</sup> *In vivo* microdialysis measurements or fast-scan voltammetry demonstrate that natural rewards and rewarding drugs promote an efflux of dopamine in the NAc.<sup>63,64</sup> The NAc shell appears to be more important for drug reward than the core.<sup>62,65</sup> It has been suggested that NAc neurons signal reward value and participate in behavioral decision making.<sup>2,66-69</sup>

### Overlapping neural circuits for pain and reward

Neural circuits mediating pain and pleasure (reward) are anatomically and functionally interconnected.<sup>46,49</sup> Neuroimaging studies in humans have identified a number of brain regions, including the NAc, rACC, and amygdala, that are implicated in both pain perception and reward processing.<sup>46,49</sup> Functional magnetic resonance imaging (fMRI) studies<sup>70</sup> show decreased activation of the NAc by the onset of an acute noxious stimulus (aversion) and increased activation by the stimulus offset (reward). Accumulating preclinical and human neuroimaging data support a crucial role for dopamine neurotransmission in modulating pain.<sup>2,71-77</sup> Disruption of dopamine signaling may underlie some chronic pain conditions including fibromyalgia and chronic orofacial pain.<sup>74,75,77</sup> Human studies, therefore, implicate the mesolimbic dopaminergic reward pathway in processing both pain and reward associated with pain relief. Accordingly, we have demonstrated in rats with incisional injury<sup>29</sup> that relief of ongoing pain with peripheral nerve block activates the mesolimbic dopaminergic reward pathway, and that such activation may underlie the motivational response to seek relief.<sup>24</sup>



### **Pain relief–induced CPP depends on activation of dopaminergic neurons in the ventral tegmental area**

As outlined above, positively reinforcing stimuli, such as natural and drug rewards, exert their function by activating dopaminergic neurons in the VTA. We have demonstrated that, in rodents, activation of these neurons is also necessary for negatively reinforced behavior following relief of ongoing pain.<sup>24</sup> Using immunohistochemistry, we have shown that in rats with incisional injury of the hind paw, pain relief with PNB resulted in an increase in the number of cFos-expressing neurons, suggesting activation of these cells. A significant increase was observed in more posterior regions of the VTA at levels  $-5.8$  to  $-6.3$  mm from the bregma. Colabeling of brain sections for cFos and tyrosine hydroxylase, a marker of dopaminergic neurons in the VTA, demonstrated that pain relief specifically activated cFos in tyrosine hydroxylase-positive (dopaminergic) neurons of the VTA. Importantly, in incised rats, inactivation of the VTA by microinjection of lidocaine or selective inhibition of dopaminergic neurons by intra-VTA administration of baclofen impeded the expression of CPP to pain relief induced by peripheral nerve block. In contrast, naloxone administration into the VTA before peripheral nerve blockade did not inhibit the PNB-induced CPP in the rats with incisional surgery, suggesting that endogenous opioids in the VTA do not mediate CPP to nerve block-induced pain relief. Thus, VTA dopaminergic neurons are essential for CPP resulting from peripheral nerve block in incisional pain.

### **Pain relief–induced CPP depends on dopamine release in the nucleus accumbens**

The primary projection target of dopaminergic VTA neurons is the NAc, where release of dopamine mediates the rewarding and motivational effects of natural and drug rewards. Accordingly, in the rat model of postsurgical pain, *in vivo* microdialysis studies in awake and freely moving rats showed that pain relief with peripheral nerve block resulted in release of dopamine in the NAc shell.<sup>24</sup> Dopamine efflux was observed during the 120 min following pain relief with a peak between 30 and 60 min. Importantly, no increase in extracellular dopamine levels was observed following PNB in sham-operated animals or when PNB was given four days postincision. This observation corresponds with the time course of ongoing pain determined by behavioral CPP studies. The role of dopamine signaling in the NAc was corroborated by CPP experiments. In rats with incision, CPP to pain relief with peripheral nerve block was prevented by inactivation of the NAc with lidocaine microinjection. Additionally, specific blockade of dopamine signaling in the NAc by microinjection of the nonselective dopamine receptor antagonist flupenthixol<sup>78</sup> also prevented pain relief-induced CPP. This directly demonstrates that dopaminergic transmission in the nucleus accumbens is required for the CPP that results from pain relief.

It should be noted that the magnitude of NAc dopamine release elicited by pain relieving treatments is lower than NAc dopamine release resulting from strong positive reinforcers (i.e., approximate dopamine release is 60% over baseline for PNB-elicited pain relief in rats with incisional injury<sup>24</sup> and 200% and 500%, respectively, with doses of systemic morphine and cocaine that produce single-trial CPP in naïve rats). Activation of reward circuits by pain relief may therefore be very selective and comparable to the engagement of the reward pathway by natural rewards (i.e., reward from relief of aversion).<sup>64</sup> The purpose of natural rewards is to restore homeostasis, and they are usually not sufficient to induce addiction.

This conclusion is supported by the many pain-relieving drugs that are not abused or addictive (e.g., NSAIDs, gabapentinoids). Opiates produce pain relief in injured states but are also rewarding (reinforcing) in noninjured states, possibly leading to addiction. It has been suggested that reinforcing effects of opiates may be suppressed in chronic pain states and indeed, morphine-induced NAc dopamine release was inhibited in rats with inflammatory pain.<sup>79</sup> Thus, the analgesic and reinforcing effects of opiates may have different value in painful and naive states, reflected in differential activation of the reward circuits. Further investigation is required to determine whether doses of morphine that produce pain relief in animals with acute or chronic pain or positive reinforcement in uninjured animals may be differentiated.

## Summary

There is an unmet need in preclinical pain research for behavioral testing methods that more accurately model aspects of pain that are likely to be important to patients. The aversiveness of pain is the most bothersome clinical problem, but difficult to evaluate in animals. Operant behavioral methods are better suited to investigate features of the complex human pain experience, including aversiveness, because, unlike reflex pain measures, they depend on cerebral pain processing. Here we review one operant method, CPP, and demonstrate that this approach can capture the presence of spontaneous or ongoing pain (including neuropathic, inflammatory, and nociceptive) in animals. Consistent with previous reports,<sup>11</sup> we show that ongoing pain differs from evoked hypersensitivity in the time of onset, duration, and severity of symptoms, as well as in the effectiveness of specific pharmacological modulation. Consequently, treatments that relieve evoked pain may not be effective for sustained pain. Such information is valuable in determining whether a new mechanism should be advanced to clinical study and how such studies should be performed.

Pain unpleasantness (aversiveness) is an important component of sustained nonevoked pain often experienced by chronic pain patients. Neural circuits and mechanisms that influence cerebral processing of pain are not well understood. CPP, as an indirect measure of pain aversiveness in laboratory animals, can be used to investigate these supraspinal mechanisms. We used CPP in rats with postsurgical pain to demonstrate the role of the mesolimbic reward pathway in pain relief elicited by peripheral nerve block. We showed that (1) peripheral nerve block one day following hind paw incision in rats produces CPP; (2) the VTA and NAc, key brain regions of the mesolimbic dopamine reward circuitry, are necessary for pain relief-induced CPP; (3) pain relief results in the activation of dopaminergic neurons in the VTA; and (4) pain relief promotes dopamine efflux in the shell region of the NAc. Thus, similar to natural rewards, relief from pain activates dopaminergic neurotransmission in the mesolimbic reward circuit. Engagement of this circuit appears to be necessary for motivated behavior, including seeking relief from ongoing pain. Importantly, the mesolimbic reward circuits are conserved across mammalian species including rodents; thus the findings of these studies are likely relevant to human pain. Indeed, fMRI studies in healthy volunteers demonstrate positive fMRI signal in the NAc at the offset of an acute thermal stimulation (pain relief).<sup>70</sup> In addition, positron emission tomography (PET) imaging with the dopamine receptor antagonist [<sup>11</sup>C]raclopride showed activation of dopamine signaling in the NAc following placebo analgesia.<sup>80</sup>

In conclusion, operant measures of pain, including CPP, may improve our understanding of the cerebral mechanisms and circuits underlying the affective component of pain and the reward of pain relief. Additionally, operant techniques may help improve translation of potential mechanisms to new pain therapies.

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**Table 1**  
**Conditioned place preference to pain-relieving treatments have been demonstrated in multiple models of experimental nociceptive, inflammatory, or neuropathic pain**

Experimental pain model	Pain relieving treatment	Y/N	Reference
Spinal nerve ligation	Spinal clonidine	Y	6
	Spinal $\omega$ -conotoxin	Y	6
	Spinal adenosine	N	6
	RVM lidocaine	Y	6, 19,22
Spared nerve injury	RVM lidocaine	Y	6
Sciatic axotomy	Spinal clonidine	Y	21
	RVM lidocaine	Y	21
Osteoarthritis (MIA)	Spinal clonidine	Y	20
	Intra-articular lidocaine	Y	23
	Contralateral intra-articular lidocaine	N	23
Inflammation (CFA)	Spinal clonidine	Y	17
	Popliteal fossa lidocaine	Y	17
Incision injury (hindpaw)	Popliteal fossa lidocaine	Y	24
	Contralateral popliteal fossa lidocaine	N	24

Note: The table summarizes whether CPP was or was not (Y/N) observed in a given pain model following the indicated treatment; MIA: monosodium iodoacetate; CFA: complete Freund's adjuvant; RVM: rostral ventromedial medulla.

**Table 2**  
**Blockade of CPP to a rapidly acting treatment by pain relieving pretreatments**

Experimental pain model	CPP to	Pain relieving pretreatment	Y/N	Reference
Spinal nerve ligation	RVM lidocaine	TRPV1 desensitization by RTX	Y	19
	RVM lidocaine	Ablation of spinal NK1 neurons	Y	19
	RVM lidocaine	NPY blockade in <i>N. gracilis</i>	N	19
	RVM lidocaine	rACC lesion	Y	21
	RVM lidocaine	rACC ZIP	Y	22
Osteoarthritis (MIA)	Intra-articular lidocaine	Diclofenac; p.o.	N	23
	Intra-articular lidocaine	TRPV1 antag. AMG9810; i.p.	N	23
	Intra-articular lidocaine	TRPA1 antag. HC030031; p.o.	N	23
Inflammation (CFA)	Popliteal fossa lidocaine	TRPV1 desensitization by RTX	Y	17
	Popliteal fossa lidocaine	TRPV1 antag. AMG9810; i.p.	N	17

Note: The table summarizes whether CPP was or was not (Y/N) blocked by the indicated pretreatment; TRPV1: transient receptor potential cation channel subfamily V member 1; RTX: resiniferatoxin; NK1: neurokinin 1; NPY: neuropeptide Y; rACC: rostral anterior cingulate cortex; ZIP:  $\zeta$ -inhibitory pseudosubstrate peptide.