Published in final edited form as:

Pain. 2014 August ; 155(8): 1659–1666. doi:10.1016/j.pain.2014.05.018.

Activation of mesocorticolimbic reward circuits for assessment of relief of ongoing pain: a potential biomarker of efficacy

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Abstract

Preclinical assessment of pain has increasingly explored operant methods that may allow behavioral assessment of ongoing pain. In animals with incisional injury, peripheral nerve block produces conditioned place preference (CPP) and activates the mesolimbic dopaminergic reward pathway. We hypothesized that activation of this circuit could serve as a neurochemical output measure of relief of ongoing pain. Medications commonly used clinically including gabapentin and non-steroidal anti-inflammatory drugs (NSAIDs) were evaluated in models of post-surgical (one day following incision) or neuropathic (14 days following spinal nerve ligation, SNL) pain to determine if the clinical efficacy profile of these drugs in these pain conditions was reflected by extracellular dopamine (DA) release in the nucleus accumbens (NAc) shell. Microdialysis was performed in awake rats. Basal DA levels were not significantly different between experimental groups and no significant treatment effects were seen in sham-operated animals. Consistent with clinical observation, spinal clonidine produced CPP and produced a dose-related increase in net NAc DA release in SNL rats. Gabapentin, commonly used to treat neuropathic pain produced increased NAc DA in rats with SNL, but not in animals with incisional, injury. In contrast, ketorolac or naproxen produced increased NAc DA in animals with incisional, but not neuropathic, pain. Increased extracellular NAc DA release was consistent with CPP and observed selectively with treatments commonly used clinically for post-surgical or neuropathic pain. Evaluation of NAc DA efflux in animal pain models may represent an objective neurochemical assay that may serve as a biomarker of efficacy for novel pain-relieving mechanisms.

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Conflict of interest statement: The authors report no conflict of interest.

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Keywords

pain; pain relief; biomarker; dopamine; nucleus accumbens; neuropathic pain; post-surgical pain; rats

1. Introduction

In spite of significant advances in understanding of the neurobiology of pain, few therapies based on novel mechanisms have advanced to clinical practice [47]. Currently available therapeutic options often demonstrate inadequate efficacy at tolerated doses or are not optimal for treatment of chronic non-malignant pain due to undesirable side effects [2; 13; 30; 45]. While many factors contribute to difficulties in discovery of improved therapeutics, one hurdle has been assessment of affective dimensions of pain in the preclinical setting [28; 49]. The development of a preclinical biomarker [1] for pain relief would be highly desirable.

Commonly used output measures from preclinical models have largely focused on modulation of behavioral responses to sensory stimulation, a relevant and important component of pain (i.e., allodynia) that is observed in some patients. Assessment of affective dimensions of pain, however, has been challenging and, until recently, few approaches were in use, (see [31] for review). Preclinical evaluation of pain using reflexive measures differs from clinical assessment that is usually based on human self-report. This raises concerns that important components of the pain experience are not captured possibly impeding translation of new analgesics effective in rodents to patients. While the limitations of laboratory animals as models for the complex experience of human pain are acknowledged, pain is fundamentally aversive in animals and in humans [10; 50]. We hypothesized that it might be possible to neurochemically assess relief of pain aversiveness in rodents as an objective, measurable, preclinical biomarker of efficacy that could serve to improve translation to the clinic.

We have used the principle of negative reinforcement to unmask the presence of ongoing (i.e., “spontaneous”) pain in injured animals [37]. Pairing an effective pain-relieving treatment (e.g., peripheral nerve block) with a context produces place preference (i.e., conditioned place preference, CPP) in animals with incisional injury [50]. This approach has been extended to a variety of clinically effective compounds with different mechanisms that are not intrinsically rewarding in multiple preclinical pain models including neuropathic [37], arthritic [40; 52], incisional [50] and cephalic [18] pain. The resulting data demonstrated strong agreement with clinical experience with medications used in specific pain states.

In humans, relief of aversive states including pain is rewarding. Extensive neuroanatomical (e.g., insula, prefrontal cortex, anterior cingulate cortex, amygdala, striatum and periaqueductal gray) and neurochemical (e.g., dopamine (DA), opioids, cannabinoids) overlap between pain and reward pathways have been demonstrated in human imaging studies [10; 12; 39]. This led us to consider that activation of brain reward pathways by relief of ongoing pain could represent a preclinical “biomarker” of treatments producing

effective relief of either post-surgical or neuropathic pain that could also be measured clinically with brain imaging providing direct translational assessment. We studied drugs commonly used for treatment of either neuropathic or post-surgical pain including clonidine (an α_2 -adrenergic agonist), gabapentin and NSAIDs (ketorolac, naproxen).

2. Materials and Methods

2.1. Animals

Adult, male Sprague-Dawley rats (250–350 g, Harlan, Indianapolis, IN) were used. All procedures were performed in accordance with the guidelines of the Committee for Research and Ethical Issues of IASP under protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Rats were initially housed three per cage on a 12 h light-dark cycle with food and water *ad libitum*.

2.2. Surgeries

2.2.1. Intracranial NAc cannulation—Stereotaxic surgeries were performed in anesthetized rats (ketamine/xylazine 80/12 mg/kg, i.p.; Western Medical Supply, Arcadia, CA/Sigma, St. Louis, MO) according to the brain atlas. A single guide cannula (AG-8, EICOM, San Diego, CA) was implanted into the left NAc shell (AP: bregma +1.7 mm; ML: midline –1.0 mm; DV: skull –6.0 mm). Stainless steel dummy cannulas were inserted to keep the guide cannula free of debris. After surgery, rats were housed individually and allowed to recover for 7 days. The placement of the guide cannula was verified histologically post-hoc. Data from the animals with misplaced cannula were discarded (<5%).

2.2.2. Intrathecal (i.th.) catheterization—While under ketamine/xylazine (80/12 mg/kg, i.p.) anesthesia, some groups of rats were implanted with i.th. catheters (polyethylene 10, 7.5 cm) through atlanto-occipital membrane extended to the lumbar spinal cord for drug administration as described previously [73]. Animals were allowed to recover for 7 days. Animals showing motor deficits or distress by the i.th. catheters were sacrificed (about 10%). The injection volume was 5 μ l treatment drug followed by a 0.5 μ l air bubble and a 10 μ l saline flush.

2.2.3. Hind paw incision—Incision injury of the skin plus deep tissue, including fascia and underlying muscle was performed as described by Brennan and colleagues [14]. Rats were anesthetized with 2% isoflurane, a 1-cm longitudinal incision was made through the skin of the left hindpaw and the plantaris muscle was elevated and incised longitudinally. The cut skin was stitched with two 5-0 nylon sutures and the wound site treated with neomycin. Sham animals were anesthetized and the left hind paw was cleaned, but no incision was made.

2.2.4. L5/L6 spinal nerve ligation (SNL)—The surgical procedure for L₅/L₆ SNL was performed according to Kim and Chung [36]. Briefly, anesthesia was induced with 5% and maintained with 2% isoflurane in air. A 2 cm midline incision was made and the lumbar 5 and 6 spinal nerves (L₅/L₆) were exposed and tightly ligated with 4-0 silk suture. The

incision was closed and the animals were allowed to recover for 14 days. Sham-operated control rats were prepared in an identical manner except that the L₅/L₆ spinal nerves were not ligated. The behavior of the rats was monitored carefully for any visual indication of motor disorders or change in weight or general health. Animals that had motor impairment or failed to show tactile or thermal hypersensitivity after the injury were excluded from further testing (about 10%).

2.3. *In vivo* microdialysis and HPLC quantification of dopamine

Microdialysis was done in awake, freely moving animals [50]. The microdialysis probe (AI-8-2, EICOM, San Diego, CA) was inserted into the NAc with 2 mm semipermeable membrane (MW cutoff: 20 kDa) projecting beyond the guide cannula and perfused at 1.25 µl/min with artificial cerebrospinal fluid (aCSF: 147.0 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl₂ and 1.2 mM CaCl₂). After a 90 min washout period, 2 baseline and 3–6 treatment fractions (30 min/fraction) were collected into pre-chilled (4°C) amber Eppendorf tubes containing 1.0 µl 40x antioxidant solution (6.0 mM L-cysteine, 2.0 mM oxalic acid and 1.3% w/v glacial acetic acid) [34]. Three or six fractions post-dose were collected for treatments with fast (i.th. or i.v.) or slow (p.o.) kinetics, respectively. All rats were then injected with cocaine (20 mg/kg, i.p.) and dialysates collected for additional 60 min.

Fractions were analyzed using Agilent 1100 HPLC system (Agilent, USA) with a 5020 guard cell, MD-150 column and Coulochem III 5014B electrochemical detector (ThermoFisher; USA) at ambient temperature. The guard cell was set at 350 mV, Electrode1 at –150 mV and Electrode2 at 250 mV. A standard curve was produced from 6 serial dilutions of DA (1.25 – 40 pg) in 20 µl aCSF plus antioxidant cocktail. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the formulas: LOD = 3.3 (SDr/S); LOQ = 10 (SDr/S); where the standard deviation of the response SDr (SD of y-intercepts of regression lines) and the slope of the standard curve S were determined from the measurements of 10 independent standard curves. The LOD and LOQ for DA were determined to be 0.286 and 0.868 pg on column, respectively. The linearity of DA peaks was also validated. The integration of the DA peaks from HPLC chromatograms was performed by an experimenter blinded to the treatment groups.

DA concentrations in microdialysates were expressed as pg/µl. The percent change of the corresponding baseline level was calculated to normalize the variations of individual rats and allow for multiple comparisons. The data of Percent Change from Baseline (PCB) were then converted to area under the time effect curve (AUC) to reflect the integrated change of the treatments. Rats that had basal DA levels below limit of quantification (LOQ) in the dialysates, incorrect cannula placement, uneven baselines (defined as >50% difference in DA concentrations between the two baseline fractions), or failed to demonstrate an increase of > 100% over baseline levels post cocaine administration were excluded from data analysis (approximately 10%).

2.4. Behavioral tests

2.4.1. Evaluation of tactile and thermal thresholds—The withdrawal threshold of the hindpaw was measured in response to probing of the plantar surface with a series of

calibrated von Frey filaments (Stoelting, Wood Dale, IL) in logarithmically spaced increments ranging from 0.41 to 15 g (4–150 N). Each filament was applied perpendicularly to the plantar surface of the left hindpaw of rats held in suspended wire-mesh cages. Withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength (“up and down” method), analyzed using a Dixon nonparametric test and expressed as the mean withdrawal threshold [17; 19; 37; 57]. The withdrawal latency of the hindpaw to an infrared radiant heat source was performed as previously described [16; 50; 72]. Rats were allowed to acclimate within a Plexiglas enclosure on a clear glass plate for 30 min. An infrared heat source was directed on the glass plate underneath the plantar surface of the left hindpaw. A motion detector halted both lamp and timer when the paw was withdrawn. Baseline latencies were established around 20 sec to allow a sufficient window for the detection of possible hyperalgesia. A maximal cutoff of 33 sec was used to prevent tissue damage. The experimenter was blinded to the experimental groups and treatments.

2.4.2. Conditioned place preference (CPP) procedures—A single trial conditioning protocol was used for CPP as previously described [37; 40; 50]. Briefly, on baseline day, rats had free access to all three CPP chambers with differing visual, olfactory and tactile cues for 15 min. Time spent in each chamber was determined through an automated system with the Anymaze program (Stoelting, USA) and the animals were assigned to different groups counterbalanced according to the baseline to ensure no pre-conditioning bias. Following baseline, some rats received incision or sham surgeries and were returned to their home cages overnight. On conditioning day (24 h post-incision or 14 days post-SNL), rats received a vehicle injection in the morning and were immediately (within 2 min) placed into the appropriate pairing chamber for 30 min. Four hours later, rats received a drug injection and were immediately placed into the opposite chamber for 30 min. On test day, 20 h following the afternoon pairing, rats were placed in the CPP box with access to all chambers and behavior was recorded for 15 min for analysis for chamber preference. Difference scores were calculated as test time minus baseline time spent in the drug-paired chamber. Animals that had biased baseline (i.e., <20% or >80% of time spent in one chamber on Day 1) or failed to explore during the testing day (<10 sec exploration in two out of the three chambers on Day 3) were excluded from the data analysis (total less than 20%).

2.5. Drug administration

Intravenous injections were performed through tail veins with 25 G needles during transient, gentle restraint. Clonidine hydrochloride (Tocris, Ellisville, MO), ketorolac tromethamine (Cayman Chemical Company, Ann Arbor, MI) and cocaine (obtained from NIDA) were dissolved in saline (0.9% NaCl). Naproxen sodium and gabapentin were purchased from Sigma and dissolved in deionized water. The dose volume for oral gavage was 4 ml/kg, 5 μ l/rat for i.th. injections and 1 ml/kg for i.v. or i.p. injections.

2.6. Statistical analysis

All results were expressed as mean $\pm$ SEM. Statistical analyses were calculated using GraphPad Prism 5 software. The AUC of DA PCB was calculated with JFlashcalc software developed by Dr. Michael Ossipov (www.u.arizona.edu/~michaelo). Unpaired t-test (two-tailed) was used when only two groups were compared; the t and P values are reported in the

Results section. For three or more group comparisons, One-Way ANOVA *post-hoc* Tukey's multiple comparisons test was used for between group comparisons. For evoked sensory threshold analysis, repeated measure Two-Way ANOVA *post-hoc* Sidak's multiple comparisons test was used to compare the treatment and time effect among groups. The $F_{(DFn,DFd)}$ and P values are reported for the ANOVA tests; *post hoc* comparison is reported as "significant" or not without the actual P value. For CPP experiments, paired t-test (two-tailed) was used to analyze the difference scores of test (post-conditioning) minus baseline; the t and P values are reported. Significance was set at $P < 0.05$.

3. Results

3.1. Basal levels of extracellular DA in NAc shell in uninjured or injured rats

We measured extracellular DA levels in the NAc shell via *in vivo* microdialysis in awake rats. Basal NAc DA levels were not significantly different in sham-operated, in nerve-injured (day 14 post-injury) or in incised (day 1 post-injury) groups ($t=0.455$, $P=0.65$ incision vs. sham; $t=1.66$, $P=0.10$ SNL vs. sham, Table 1).

3.2. Effects of spinal clonidine on NAc DA levels in SNL rats

Consistent with clinical efficacy of intrathecal (i.th.) clonidine in relieving neuropathic pain in patients [23; 25], we previously showed that i.th. administration of clonidine blocked evoked hypersensitivity in animals with spinal nerve ligation (SNL) neuropathic injury and also produced CPP selectively in injured animals [37]. These observations were extended here by determining extracellular DA levels in the NAc shell via *in vivo* microdialysis in SNL or sham-operated rats following treatment with spinal clonidine. Spinal clonidine (3, 6 and 10 μ g) produced an apparent dose-related effect on DA efflux (Fig. 1). However, only the highest dose (10 μ g) showed a statistically significant difference from vehicle ($F_{(5, 42)}=4.48$, $P=0.002$, Fig 1. A & B). The time-dependent increases in NAc DA levels peaked at 30–60 min post-dose (i.e. fraction 2) in nerve-injured rats. The peak of DA efflux was observed at fraction 2, consistent with the approximately 10 min requirement for dialysate to reach the collection vials from the outlet branch of the microdialysis probes, a consequence of the slow flow rate and length of tubing. Spinal clonidine (10 μ g) had no effect in sham-operated animals.

3.3. Effects of oral gabapentin on evoked thresholds and NAc DA levels in rats with neuropathic or post-surgical pain

Gabapentin (300 mg/kg, p.o.) increased NAc DA efflux in SNL, but not sham-operated, rats ($F_{(3, 25)}=4.292$, $P=0.014$, Fig 2. A & B). The DA percent change from baseline (PCB) peaked at 150–180 min post-dose (Fraction 6). Oral gabapentin also reversed tactile hypersensitivity at 2 and 4 h post-dose in SNL rats ($F_{(1, 14)}=194.2$, $P<0.0001$, Fig 2. C). No significant change of NAc DA levels ($F_{(3, 23)}=0.827$, $P=0.49$) or tactile thresholds ($F_{(1, 60)}=2.80$, $P=0.10$) was observed with this dose of gabapentin in animals with incisional injury, or in sham animals (Fig 2. D, E & F).

3.4. Effects of non-steroidal anti-inflammatory drugs (NSAIDs) on CPP, evoked thresholds and NAc DA levels in rats with neuropathic or post-surgical pain

Systemic ketorolac (10 mg/kg, i.v.) induced robust preference to the drug paired chamber selectively in incised rats; the difference score was 225.5 ± 57.7 and -16.5 ± 45.7 s in incisional ($t=-3.91$, $P=0.0045$) and sham ($t=0.36$, $P=0.73$) rats, respectively (Fig 3. A). Intravenous ketorolac produced a significant ($F_{(1, 15)}=11.41$, $P=0.004$) increase in withdrawal threshold 20 minutes after injection in incised rats ($F_{(1, 15)}=11.41$, $P=0.004$, Fig 3. B), and significantly increased NAc DA levels in rats with incisional injury ($F_{(3, 30)}=4.27$, $P=0.013$), but not sham treatment (Fig 3. C & D). The DA PCB peaked at 30–60 min post-dose (Fraction 2). Intravenous saline treatment did not produce significant change in DA levels. Spinal ketorolac (50 μ g, i.th.) did not produce CPP ($t=-1.47$, $P=0.16$) or change in evoked pain ($F_{(1, 7)}=0.25$, $P=0.63$) or DA levels ($F_{(3, 20)}=0.59$, $P=0.63$) in incised rats (Fig 3. I, J, K & L). Intravenous ketorolac (10 mg/kg) did not induce CPP ($t=1.3$, $P=0.22$) and had no significant effect on tactile hypersensitivity ($F_{(1, 18)}=1.28$, $P=0.27$) or NAc DA levels ($F_{(3, 25)}=0.37$, $P=0.77$) in SNL or sham-operated rats (Fig 3. E, F, G & H).

The effect of a second NSAID, naproxen, which is formulated only for oral administration, on DA efflux was also examined. Naproxen (100 mg/kg, p.o.) increased NAc DA efflux in incised ($F_{(3, 25)}=6.42$, $P=0.002$), but not sham, rats (Supplemental Fig 1. A & B). The DA PCB peaked at 150–180 min post-dose (Fraction 6). Oral naproxen also partially reversed tactile ($F_{(1, 8)}=14.71$, $P=0.005$, Supplemental Fig 1. C) and thermal ($F_{(1, 8)}=9.42$, $P=0.015$, Supplemental Fig 1. D) hypersensitivity at 2 and 3 h post-dose in incised rats. However, this dose of naproxen had no significant effect on NAc DA levels ($F_{(3, 21)}=0.13$, $P=0.94$) or tactile hypersensitivity ($F_{(1, 9)}=3.58$, $P=0.09$) in SNL or sham-operated animals (Supplemental Fig 1. E, F & G).

3.5. Parallel changes between the CPP Difference Score and DA release in NAc by pain-relieving treatments

We determined if a correlation could be demonstrated between NAc DA efflux and place preference difference scores with the treatments described here in experimental post-surgical and neuropathic pain, as well as with other treatments in multiple pain models that we have previously reported including incisional pain on post-operative days 1 and 4 with lidocaine-induced peripheral nerve block and cephalic pain induced by dural inflammation with i.v. CGRP antagonist (α CGRP₈₋₃₇) [18; 50]. We used the peak NAc DA efflux as this was independent of collection fraction and pharmacokinetics of the administered treatment. We found that the neurochemical and behavioral output measures form 2 clusters, with one group showing low CPP scores and low DA efflux and the second showing high CPP scores and high DA efflux (Fig 4).

4. Discussion

We used a “reverse translation” strategy to explore whether drugs that have clinical efficacy in either neuropathic or post-operative pain would demonstrate increased nucleus accumbens (NAc) DA efflux in preclinical models of these pain states. We hypothesized that NAc DA efflux could represent an objective, quantifiable neurochemical output

measure reflecting relief of ongoing pain, independent of pharmacokinetics important in behavioral assessment, providing a new way to evaluate pain mechanisms.

Pain is aversive across species and produces motivational drive to seek relief [49]. Human [9; 11] and animal [50] data suggest that pain relief is rewarding [12]. Human imaging studies have shown that the NAc is activated by acute pain offset [7; 9] as well as by placebo analgesia [62]. Activation of NAc by pain offset was demonstrated by fMRI in rats [11]. Activation of reward-related pathways likely underlies the motivation to seek relief from ongoing pain and results in negative reinforcement that can be captured with CPP [18; 37; 50; 53]. In rats with incisional injury, peripheral nerve block (PNB) increased DA efflux in the NAc shell and CPP was blocked by administration of intra-NAc dopaminergic antagonist [50]. Similarly, in rats with cephalic pain induced by sterile dural inflammation, intravenous calcitonin gene related peptide (CGRP) antagonist, α CGRP₈₋₃₇, produced CPP and elicited NAc DA release [18]. Importantly, PNB or CGRP antagonist only produced these effects in injured rats, a finding replicated with effective treatments in multiple models [18; 37; 50; 53]. These findings are extended here by aligning the outcome measures of DA efflux, CPP and tactile hypersensitivity from animals with nerve injury or incisional pain using clinically relevant drugs within the same study. The data from these outcome measures with pharmacological treatments commonly employed clinically in these pain conditions support the potential translational utility of NAc DA efflux as a neurochemical output measure of efficacy of relief of ongoing pain.

The NAc encodes salience and valence of reward and aversive signals [10; 51; 69]. Two major subdivisions of NAc, core and shell, have been identified in rats and primates [8; 32; 43; 76]. The shell was targeted since previous reports and our own finding indicate that relief of acute or ongoing pain induces DA efflux in this NAc region in rats [15; 50]. Acute pain stimuli decrease fMRI BOLD signals [10] and increase DA release [61] in NAc in healthy volunteers, as well as in animals [3; 38; 59]. After sustained application of a painful stimulus, the BOLD signals return to baseline level [9]. The DA reward pathway encodes associative learning and valuation [15] and thus likely responds to changes in pain state. Consistent with this, and within the temporal limitations of our microdialysis technique, differences in basal extracellular NAc DA levels were not detected among incised, SNL or sham-operated rats at 24 h or 14 d post injury. However, increased NAc DA was observed with analgesic treatments in injured animals. Spinal clonidine, an α_2 -adrenergic receptor agonist, is effective in human neuropathic pain [24; 44; 60]. Here, spinal clonidine produced a time-related increase in NAc DA levels only in injured (i.e., SNL) animals, consistent with our previous report of CPP and inhibition of evoked hypersensitivity [37]. The increase in NAc DA appeared to be dose-related although only the highest dose achieved statistical significance from baseline. The actions of spinal clonidine on NAc DA efflux are suggestive of partial blockade of afferent input but this possibility requires further investigation.

Gabapentin provides pain relief in about one third of neuropathic pain patients with a number-needed-to-treat (NNT) of 5.8 [48], but is inferior to commonly used analgesics in acute postoperative pain (NNT=11) [65]. High oral doses of gabapentin are often used in humans due to non-linear dose absorption [6; 58; 64; 70]. Preclinical studies also use high doses of p.o. gabapentin (100–300 mg/kg) [33; 46; 54]. Here, gabapentin (300 mg/kg, p.o.)

reduced evoked hypersensitivity and elicited NAc DA efflux in SNL, but not incised, rats. Our studies indicate that gabapentin (100 or 300 mg/kg, p.o.) is also effective in the CPP paradigm and elicits NAc DA release in SNL animals (unpublished observations). This dose of gabapentin produces motor impairment [27], likely due to sedation, and impacts interpretation of results obtained with evoked somatosensory endpoints. However, in CPP studies, conditioning is done with the drug but testing occurs when no drug is present, mitigating this potential concern. Additionally, DA efflux is not dependent on motor activity.

NSAIDs, ineffective for neuropathic pain [20], are useful in managing post-surgical pain [5; 66]. In humans, ketorolac is administered orally, intramuscularly, intravenously or topically to manage moderate to severe postoperative pain conditions that otherwise require opioids [26; 29; 41; 71; 74; 75]. The intravenous formulation produces rapid effects allowing direct evaluation of CPP in rats and possible correlation with NAc DA. Intravenous ketorolac transiently reversed hypersensitivity and produced CPP and NAc DA efflux in incised animals; this treatment had no effect in SNL animals. The apparent discordance between modulation of evoked hypersensitivity and CPP response may reflect differences in mechanism and time-course of hypersensitivity and ongoing pain. Post-surgical patients frequently report extended sensitivity to touch or movement-evoked pain (tenderness) while otherwise experiencing satisfactory post-operative pain relief, even with opioids [68].

Unlike systemic administration, spinal ketorolac was not active in human experimental pain [22] or in patients with post-surgical or chronic pain [21]. Accordingly, spinal ketorolac did not elicit CPP or DA efflux in animals with incision. Oral naproxen produced a delayed increase in NAc DA release, consistent with its route of administration, and partially blocked evoked hypersensitivity in incisional, but not SNL, animals.

Collectively, we found parallel changes between DA efflux in the NAc shell and the CPP difference score supporting the possibility that these measures may be interchangeable for detection of relief of ongoing pain. Increased NAc DA release and CPP may result from modulation of nociceptive pathways, affective dimensions of pain, or both. Importantly, increases in NAc DA release that are produced by a treatment in a specific pain model may reflect efficacy that is sufficiently meaningful to elicit motivated behavior in injured animals and the reverse may also be inferred. Thus, this neurochemical output measure may have translational value to human pain conditions where affective dimensions appear to be most relevant [55; 56]. Critically, none of the treatments tested here produced effects in uninjured animals consistent with the lack of intrinsically rewarding properties of these drugs in humans. Caution must be taken in interpretation of the data if the drugs tested are intrinsically rewarding in uninjured animals (e.g., opioids or catecholamine reuptake inhibitors).

In summary, clinically effective analgesics elicit increases in NAc DA release and motivated behavior in specific preclinical models of pain. These effects on NAc DA were only seen in injured animals treated with pain medications. Therefore, the increased extracellular levels of DA in NAc shell might serve as an objective and quantifiable neurochemical biomarker confirming the relief of ongoing pain in preclinical drug development flow schemes. Ideally,

a biomarker would demonstrate tight pharmacokinetic/pharmacodynamic correspondence. The dynamics of DA efflux in relation to drug exposure and to pain relief are currently unknown. Additionally, it is not known whether changes in DA efflux, as measured here, have sufficient time resolution to show correspondence to drug levels. Notably, the site at which drug levels would be measured are also not completely clear particularly for drugs such as gabapentin where the molecular target is uncertain. Further mechanistic investigation is required before practical implementation in drug development programs. Potential changes in levels of DA, or its metabolites, in the CSF or in plasma in relation to pain relieving treatments would be of high importance due to the relative accessibility of samples; this possibility also requires further investigation.

This concept may also be relevant to clinical investigations where activation of NAc can be determined via imaging tools following treatment with novel chemical entities and evaluated as a potential measure of pain relief complementing the subjective report of pain score. PET imaging studies have captured dopaminergic signaling in the ventral striatal area in response to pain and pain relief in humans [61; 62]. The potential translational utility of this approach is also demonstrated by the recent BOLD imaging study showing analogous activation of the NAc in rats in response to pain onset and offset as described in humans [11]. Additionally, it is noted that there are circumstances in humans where determining whether pain is present is difficult, including schizophrenia, Alzheimer's, mental retardation, elderly or young children including neonatal patients [4; 35; 42; 63; 67]. In these individuals, ongoing pain might be unmasked with imaging the activation of this circuit with a transient pain relieving intervention allowing for appropriate decisions in medical management of the patient. The identification of a biomarker for relief of pain thus has the potential to contribute to clinical evaluation of ongoing pain in patients as well as potentially facilitating rapid preclinical validation of novel mechanisms that may help in promoting the discovery of new therapies for pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We sincerely thank Dong Lu, David J. Wasiak, Janice Oyarzo, Bradley S. Silver, Naomi Goshima, Pablo Hernandez and Francesca Porreca for their technical assistance in collecting the data and Amy Porter for helpful comments on the manuscript. This study was supported by NS 066958 and DA 034975 from the NIH.

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Summary

Relief of ongoing pain may be reflected by increased dopamine efflux in the nucleus accumbens providing a neurochemical efficacy measure for evaluation of novel analgesics.

Fig 1. Change of extracellular DA levels in NAc shell in nerve injured (SNL) rats treated with spinal clonidine (top panel)

Clonidine (3, 6 or 10 μg, i.th.) dose- and time-dependently increased DA efflux in SNL, but not sham-operated, animals (A, B). The fitted curve (C) shows the dose-response of the spinal clonidine versus area under the time effect curve (AUC) of NAc DA Percent Change from Baseline (PCB). * $P < 0.05$ compared to vehicle control or sham groups ($n = 4-11$ /group).

Fig 2. Extracellular DA levels in NAc shell and evoked sensory threshold in SNL (top panel) or incisional (bottom panel) rats treated with oral gabapentin
 Systemic gabapentin (300 mg/kg, p.o.) increased DA efflux in NAc shell (A, B) and reversed tactile hypersensitivity (C) in SNL, but not sham, animals. In contrast, gabapentin did not significantly alter NAc DA levels (D, E) or tactile hypersensitivity (F) in incised animals. One-way ANOVA *post hoc* Tukey's multiple comparisons test (B, E) or Two-way ANOVA *post hoc* Sidak's multiple comparisons test (C, F) was used for statistical analysis. * $P < 0.05$ compared to vehicle control or sham groups ($n = 5-9$ /group).

Fig 3. CPP, tactile hypersensitivity and NAc DA levels in incised (top and bottom panels) or neuropathic (middle panel) rats treated with i.v. or i.th. ketorolac
Systemic ketorolac (10 mg/kg, i.v.) induced CPP (A), reversed tactile hypersensitivity (B) and increased NAc DA efflux (C, D) in incised, but not sham or SNL (E, F, G, H), animals. Spinal ketorolac (50 μ g, i.th.) did not produce effects in any of these measures in incised rats (I, J, K, L). Paired t-test (A, E, I), Two-way ANOVA *post hoc* Sidak's multiple comparisons test (B, F, J) or One-way ANOVA *post hoc* Tukey's multiple comparisons test (D, H, L) was used for statistical analysis. * $P < 0.05$ compared to vehicle control or sham groups ($n = 4-11$ /group).

Fig 4. Parallel changes between the peak increase of extracellular DA levels from baseline and CPP difference score

Treatments producing significant changes in CPP difference scores are shown as solid labels while those that were not significant are represented by half empty labels. Circle, square and diamond shapes represent SNL, incision and cephalic pain models, respectively. Two distinct groupings appear, with one cluster showing low CPP scores and DA efflux and the second showing high CPP scores and significant DA efflux. The clusters are outlined on the graph.

Table 1

Basal DA levels in injured or uninjured animals. N = number of animals in each group.

	Sham-SNL	SNL	Sham-Incision	Incision
DA Conc. (pg/ μ l)	0.181 $\pm$ 0.103	0.156 $\pm$ 0.067	0.144 $\pm$ 0.083	0.138 $\pm$ 0.066
N	54	68	44	79