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Negative Reinforcement Reveals Non-Evoked Ongoing Pain in Mice With Tissue or Nerve Injury

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Abstract: Patients with chronic pain experience spontaneous or ongoing pain as well as enhanced sensitivity to evoked stimuli. Spontaneous or ongoing pain is rarely evaluated in preclinical studies. In fact, it remains controversial whether ongoing or spontaneous pain even develops in mice after tissue or nerve injury. This study tested a hypothesis that negative reinforcement can be used to unmask the presence of pain in mice with tissue or nerve injury. We found that spinal administration of clonidine or lidocaine did not elicit conditioned place preference (CPP) in uninjured or sham-operated mice. However, these agents produced CPP in mice with chronic inflammation induced by complete Freund's adjuvant (CFA) or following L5/L6 spinal nerve ligation (SNL). These data indicate the presence of non-evoked (ie, stimulus-independent) ongoing pain in mice with chronic inflammation (CFA) or following nerve injury (SNL). In addition, this study validates the use of negative reinforcement to unmask non-evoked ongoing pain in mice. Given the existence of a large collection of transgenic and knockout mice, our data show the application of this approach to elucidate molecular mechanisms underlying non-evoked pain and to contribute to drug discovery for pain.

***Perspective:** We demonstrated the presence of non-evoked ongoing pain in mice with chronic inflammation or following nerve injury. The study also validates the use of negative reinforcement to unmask non-evoked pain in mice. We propose to apply this approach to identify molecular mechanisms and effective drugs for chronic pain.*

***Key words:** Spontaneous pain, conditioned place preference, neuropathic pain, inflammatory pain, negative reinforcement.*

Spontaneous pain is a common complaint of patients with chronic pain.[1,28,32] Although spontaneous pain, either paroxysmal or ongoing, is consistently identified as the major clinical complaint in many pain states, its detection and mechanistic evaluation present a major challenge preclinically. Current research has relied almost exclusively on reflexes from an evoked stimulus, a threshold response, whereas patients suffer from non-evoked pain that is evaluated clinically on the basis of self-reported intensity. Evoked hypersensitivity is unquestionably a concern in some clinical settings; however, most clinical trials rely on evaluation of tonic pain intensity, suggesting the possibility that measures of evoked thresholds may not accurately predict efficacy of therapies in development for the treatment of pain.[9,14,27,31] This limitation may be a major obstacle in basic and translational research to identify effective drugs for chronic pain.

Recently, negative reinforcement was employed to reveal the presence of non-evoked ongoing pain in rats.[19,29,30] This approach employed conditioned place pairing to unmask the presence of an aversive state as the result of non-evoked ongoing pain. Importantly, drugs that were not rewarding in the absence of chronic pain elicited conditioned place preference (CPP) in rats with ongoing pain by alleviation of ongoing pain.[19] This approach has also been applied to rats with lesions of the spinal cord representing central pain.[7]

Whether mice might experience spontaneous or ongoing pain after tissue or nerve injuries remains controversial. A recent study monitored home cage activity and behaviors that are thought to reflect the affective state following chronic inflammation or peripheral nerve injuries and concluded that there was no change in "quality of life" measures in mice.[34] The study failed to demonstrate behavioral evidence for ongoing pain in

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male mice after intraplantar complete Freund's adjuvant (CFA), spared nerve injury (SNI), or chronic constriction injury (CCI).[34] The current study was designed to test the hypothesis that injuries to tissues or to peripheral nerves were accompanied by spontaneous or ongoing pain in mice. We propose that negative reinforcement can be used to unmask the presence of non-evoked ongoing pain in mice with tissue or nerve injury. Specifically, we determined whether spinal administration of drugs that do not produce CPP in naïve mice would do so in the presence of injury, revealing the presence of an aversive state reflecting ongoing pain in mice. To date, whether this approach can be successfully applied to mice has not been established. If successful, it would not only offer a powerful approach to directly test our hypothesis, but also open the opportunity of genetic advantages conferred by mouse models.

Methods

Animals

Male ICR mice (20–25 g; Harlan, Indianapolis, IN) were maintained on a 14/10 hour light/dark cycle (light on 5:00 AM–7:00 PM: a standard light/dark schedule that is used by the university animal care facility) with food and water provided ad libitum before experimental procedures. All experiments were performed during the light cycle. Mice were randomly divided into experimental groups according to a computer-generated randomization list. All procedures were carried out in accordance with the International Association for the Study of Pain and the National Institutes of Health Guide for the Care and Use of Laboratory Animals after approval by the University of Illinois Institutional Animal Care and Use Committee.

Materials

CFA (.5 mg/mL *Mycobacterium tuberculosis* [H 37RA, ATCC 25177], suspended in an oil:saline [1:1] emulsion), lidocaine, clonidine, and adenosine were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade or better from commercial sources.

Drug Administration

Intrathecal injection (i.t.) was given in a volume of 5 μL by percutaneous puncture through an intervertebral space at the level of the 5th or 6th lumbar vertebra, as described previously.[5,15,33]

Conditioned Place Preference (CPP)

The CPP apparatus (San Diego Instruments, San Diego, CA) consists of 3 Plexiglas chambers separated by manual doors. A center chamber (6 1/4" W × 8 1/8" D × 13 1/8" H) connects the 2 end chambers, which are identical in size (10 3/8" W × 8 1/8" D × 13 1/8" H) but can be distinguished by texture of floor (rough versus smooth) and wall pattern (vertical versus horizontal stripes). Movement of mice and time spent in each chamber were monitored by 4 × 16 photobeam arrays and automatically recorded in San Diego Instruments CPP software.

Preconditioning was performed across 3 days for 30 minutes each day when mice were exposed to the environment with full access to all chambers. On day 3, a preconditioning bias test was performed to determine whether a preexisting chamber bias existed. In this test, mice were placed into the middle chamber and allowed to explore open field with access to all chambers for 15 minutes. Data were collected and analyzed for duration spent in each chamber. Animals spending more than 80% or less than 20% of the total time in an end chamber were eliminated (~10% of total animals) from further testing.

We used a single trial conditioning protocol in the experiments.[19] On conditioning day (day 4), mice first received vehicle control (saline, i.t.) paired with a randomly chosen chamber in the morning and, 4 hours later, either clonidine (1 μg in 5 μL saline, i.t.), lidocaine (.04 % in 5 μL saline, i.t.), or adenosine (3 μg in 5 μL saline, i.t.) paired with the other chamber in the afternoon. These doses were selected based on pilot experiments. During the conditioning, mice were allowed to stay only in the paired chamber without access to other chambers for 15 minutes immediately following saline or drug injection. On the test day, 20 hours after the afternoon pairing, mice were placed in the middle chamber of the CPP box with all doors open so animals could have free access to all chambers. Movement and duration of each mouse spent in each chamber were recorded for 15 minutes for analysis of chamber preference. Difference scores were calculated as test time – preconditioning time spent in the drug chamber.

Complete Freund's Adjuvant (CFA)-Induced Inflammatory Pain Model

Unilateral inflammation was induced by injecting 20 μL CFA into the dorsal surface of the left hindpaw (intraplantar injection), as we have previously described.[26,33] The treatment is known to induce thermal hyperalgesia and mechanical allodynia. Control mice received 20 μL of saline. Sensitivity to thermal and mechanical stimuli was tested before and after CFA injection. CPP preconditioning started 3 days before CFA injection.

Spinal Nerve Ligation (SNL)-Induced Neuropathic Pain Model

SNL was carried out as previously published.[4,18] Separate groups of 8 mice had the left L5 and L6 spinal nerves tightly ligated distal to the dorsal root ganglion but before the fibers join to form the sciatic nerve; the sham operation consisted of the same surgery but without nerve ligations. Sensitivity to thermal and mechanical stimuli was tested before and after SNL operation. Preconditioning started 10 days after SNL.

Assessment of Mechanical and Thermal Sensitivity

Sensitivity to mechanical stimulus was assessed as previously described.[4,26] Mice were allowed to acclimate for 30 minutes before probing with calibrated von Frey