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Conditioned Place Preference Reveals Tonic Pain in an Animal Model of Central Pain

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Abstract: A limitation of animal models of central pain is their inability to recapitulate all clinical characteristics of the human condition. Specifically, many animal models rely on reflexive measures of hypersensitivity and ignore, or cannot assess, spontaneous pain, the hallmark characteristic of central pain in humans. Here, we adopt a conditioned place preference paradigm to test if animals with lesions in the anterolateral quadrant of the spinal cord develop signs consistent with spontaneous pain. This paradigm relies on the fact that pain relief is rewarding to animals, and has been used previously to show that animals with peripheral nerve injury develop tonic pain. With the use of 2 analgesic treatments commonly used to treat patients with central pain (clonidine infusion and motor cortex stimulation), we demonstrate that analgesic treatments are rewarding to animals with spinal cord lesions but not sham-operated controls. These findings are consistent with the conclusion that animals with spinal cord injury suffer from tonic pain.

***Perspective:** The hallmark characteristic of central pain in humans is spontaneous pain. Animal models of central pain rely on reflexive measures of hypersensitivity and do not assess spontaneous pain. Demonstrating that animals with spinal cord injury suffer from tonic pain is important to study the etiology of central pain.*

***Key words:** Spontaneous pain, motor cortex stimulation, posterior thalamus, rat, clonidine.*

A common consequence of spinal cord injury is the development of severe, debilitating chronic pain.[1,29,36] In patients, the pain manifests with a wide range of intensities and locations. It is usually persistent in the absence of an insult (spontaneous pain), and can present as hypersensitivity to painful stimuli (hyperalgesia) and hypersensitivity to normally innocuous stimuli (allodynia).[2] The etiology of the pain is unknown and is thought to be caused by maladaptive changes in the central nervous system.

Several animal models have been developed to study central pain, many of which focused on studying pain due to spinal cord injury. In all of these models, the location, the extent, and the means to produce injury vary. Some authors use controlled spinal contusions to mimic clinical traumatic injuries.[12,26,39] Others have used ischemic lesions,[9,10] or neurotoxic chemical injection into the spinal cord,[4,37] whereas some have used cuts to sever the spinal cord (hemisection),[5,6] or localized regions in the spinal cord (cordotomy).[31,32] Most of these models rely on measures of evoked pain and hypersensitivity, such as mechanical and thermal withdrawal thresholds. However, they commonly do not attempt to quantify spontaneous pain, which is the single most common and debilitating complaint from spinal cord injury patients.[8,29] Our aim was to assess whether animals with spinal cord injury suffer from spontaneous pain.

We have demonstrated recently that localized electrolytic lesions in the anterolateral quadrant of the spinal cord result in consistent, long-lasting mechanical and

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thermal hyperalgesia.[19] Like other animal models of central pain, we relied on evoked measures of hypersensitivity to assess hyperalgesia and did not test if animals exhibit symptoms of spontaneous pain. Here, we employ a conditioned place preference paradigm described by King et al[14] to study tonic pain in animals. This approach takes advantage of the fact that pain relief is rewarding and, therefore, analgesic treatments should only be rewarding in the presence of pain.[14] We use the conditioned place preference paradigm combined with 2 treatments known to alleviate neuropathic pain (clonidine infusion or electrical stimulation of the motor cortex) to test if animals develop signs of spontaneous pain following spinal cord lesions. We demonstrate that lesioned animals, but not sham-operated controls, develop rapid preference to the analgesic treatment-paired chamber.

Methods

All procedures were approved by the University of Maryland Animal Care and Use Committee. Experiments were conducted according to institutional guidelines, federal regulations, and the guidelines of the International Association for the Study of Pain.

Protocol Overview

Twenty-eight adult female Sprague-Dawley rats (Harlan, IN) weighing 250 to 300 g, were used in this study, which was conducted over a 10-week period. Two experiments were conducted concurrently: 1) Drug group, to test the effect of analgesic drug administration (clonidine) on the conditioned place preference of animals with spinal cord injury (n = 11); and 2) Stimulation group, to test the effect of motor cortex stimulation on the conditioned place preference of animals with spinal cord injury (n = 17). In weeks 1 and 2, rats were habituated to handling and trained to stand with their forepaws on the experimenter's hand, allowing access to the hindpaws, as described in Ren.[25] During week 3, rats underwent behavioral tests to measure mechanical hindpaw withdrawal thresholds (see below). During week 4, rats underwent spinal lesion surgery to induce central pain or sham lesion surgery as a control and, for animals receiving motor cortex stimulation, to implant insulated platinum electrodes (see below). Weeks 5 and 6 involved further behavioral testing to measure mechanical hindpaw withdrawal thresholds and monitor the development of injury-related hyperalgesia. During week 7, rats in the clonidine/saline group underwent surgery to implant cannulae in the lateral ventricle for drug administration. Week 8 involved recovery from surgery and further testing of mechanical hindpaw withdrawal thresholds. The conditioned place preference protocol was conducted during weeks 9 and 10, along with further testing of mechanical hindpaw withdrawal thresholds.

Mechanical Hindpaw Withdrawal Threshold Testing

Mechanical hindpaw withdrawal thresholds were measured bilaterally using calibrated von Frey filaments (Stoelting, IL). Filaments with forces ranging from 10 to 180 g were applied to the dorsal surface of the hindpaw, based on studies demonstrating that threshold changes are more reliably and consistently detected at this site.[25] Each von Frey filament was applied 5 times to each hindpaw and the threshold was defined as the force at which the animal withdrew the paw to 3 or more of the stimuli (>50% response frequency). Animals were not restrained during testing. Rats underwent von Frey testing on 3 days in week 3 (before spinal or sham lesion surgery) to obtain baseline presurgical withdrawal thresholds, and every 7 days postlesion surgery for the duration of the study. Rats were also tested during the conditioned place preference protocol (week 10) to determine mechanical thresholds in the presence of intraventricular drug treatment or motor cortex stimulation (see below).

Surgical Procedures

Spinal Lesions

Fifteen adult female Sprague-Dawley rats underwent spinal lesion surgery, and 13 underwent sham lesion surgery during week 4 of the study. Eleven rats (n = 6 lesioned, n = 5 sham) underwent surgery to implant a cannula in the right lateral ventricle during week 7 of the study. Surgeries were conducted under strict aseptic conditions. Rats were anesthetized with ketamine/xylazine (100/8 mg/kg, ip) and placed on a thermo-regulated heating pad to maintain body temperature. For spinal lesions, a laminectomy was performed to expose the spinal cord between C6 and T2. A quartz-insulated platinum electrode (5-μm tip) was targeted unilaterally to the ventrolateral quadrant of the spinal cord, as described previously.[19,33] Current (10 μA for 10 seconds, repeated 4 times) was passed through the electrode to produce an electrolytic lesion (approximately .6 mm^3; lesion locations, .8 mm lateral from midline; depth, 2.1 mm). In some animals (n = 9), to produce larger spinal lesions, we modified our approach to produce 2 lesions, .4 mm apart (lesion locations, .8 mm and 1.2 mm lateral from midline; depth, 2.1 mm). However, the modification in the protocol had no effect on the consistency or features of the resultant hyperalgesia. Sham surgery was performed without laminectomy.

Implantation of Motor Cortex Stimulation Electrodes

In 17 animals ("stimulation group") and, concurrent with spinal lesion surgery, a longitudinal incision was made along the midline of the skull to expose bregma and lambda. The bone overlying the primary motor cortex (MI) was removed contralateral to the spinal lesion site. Custom-made epidural bipolar insulated platinum electrodes (diameter, 70 μm; exposed tip, 50 μm; distance between electrodes, 500 μm) were targeted to the MI contralateral to the site of spinal lesion using stereotaxic coordinates (A: 1.8 mm, L: 2 mm). These coordinates were obtained from pilot experiments using electrical microstimulation and from data obtained from our previously