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Correlation Between Ventral Striatal Catecholamine Content and Nociceptive Thresholds in Neuropathic Mice

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Abstract: **Neuropathic pain is characterized by persistent, intractable pain following damage or dysfunction of the nervous system. Analgesics that include central, rather than purely peripheral, targets are more effective when treating neuropathic pain, highlighting the spinal and/or supraspinal mechanisms that contribute to this aberrant pain condition. The striatum represents one of the brain regions that have been implicated in pain processing. Release of dopamine in the ventral striatum is normally associated with analgesia. Clinical and human imaging studies suggest that dopamine is disrupted in neuropathic pain patients, although the conclusions drawn from these studies are limited by their noninvasive imaging or pharmacologic approaches. In this study, we used a C57Bl/6 mouse model of neuropathic pain to describe the changes in neurotransmitter content in the striatum and their relationship to evoked pain thresholds. Striatal dopamine content negatively correlated with mechanical thresholds in sham animals. Neuropathic pain animals had reduced dopamine content that was not correlated with mechanical thresholds. In contrast, norepinephrine content was significantly increased and correlated with mechanical thresholds in neuropathic, but not sham, animals. These results describe changes in striatal signaling in neuropathic pain animals and contribute to the literature defining the role of dopamine and norepinephrine in mediating sensory thresholds in healthy and neuropathic pain states.**

Perspective: ***Results show significant loss of ventral striatal dopamine in neuropathic pain conditions, and the relationship of ventral striatal catecholamines to pain thresholds is changed in neuropathic pain. These results complement human imaging studies and provide evidence that chronic pain alters the function of reward systems.***

Key words: *Basal ganglia, catecholamines, chronic pain, neuralgia, pain thresholds.*

Neuropathic pain is a chronic pain condition resulting from damage or dysfunction of the nervous system. The chronic, intractable nature of the pain results in adaptations in the nervous system that contribute to the chronicity of this condition and undermines the efficacy of classical analgesics, such as opioids.[26] The ventral striatum receives inputs from limbic structures such as the amygdala, hippocampus, and thalamus, as well as from the mesolimbic dopamine system originating in the ventral tegmental area (VTA). Accordingly, the ventral striatum is a critical system in attributing incentive salience and emotional valence to sensory stimuli in the environment, including pain.[4] However, the ventral striatum is also directly involved in modulating pain itself. Lesions of striatal neurons enhance nociception and interfere with opioid analgesia.[23] Conversely, activation of the ventral striatum, whether by electrical stimulation or local injection of a dopamine receptor 2 agonist, produces analgesia.[28,29] How signaling within the ventral striatum changes in neuropathic pain, and the influence these changes have on pain, is a question that has remained minimally addressed in the literature.

Clinical and human imaging studies suggest that dopaminergic signaling in the striatum is perturbed in neuropathic pain. Human imaging studies using positron emission tomography (PET) found that fibromyalgia patients have reduced presynaptic [^{18}F]6-fluoro-L-dopa reuptake and increased dopamine receptor availability.[16-18,33] Patients with burning mouth syndrome and

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atypical facial pain exhibited higher dopamine receptor 2 availability in the dorsal striatum when compared to healthy age- and sex-matched controls.[15,16] These studies suggest that neuropathic pain patients exhibit either lower endogenous dopamine or increased dopamine receptor expression in the striatum. Drugs that restore dopaminergic tone, such as the dopamine reuptake inhibitor bupropion, can be effective treatments for neuropathic pain.[25] Further, Parkinson's disease, a condition characterized by loss of dopaminergic neurons in the nigrostriatal pathway, is sometimes associated with lowering of pain thresholds, which can be restored with L-dopa treatment.[12-14,31] In fact, pain is the most common sensory disturbance in Parkinson's disease and is often comorbid with neuropathic pain conditions.[6,24,27]

Although the studies presented above clearly point to a disruption in striatal dopamine in neuropathic pain, most rely on imaging techniques with radiolabeled ligands that bind to dopamine receptors. These studies are limited because changes in dopamine receptor availability could reflect a difference in receptor number or a change in endogenous dopamine occupancy. Further, it is difficult to determine whether dopaminergic hypofunction is a result of neuropathic pain or a premorbid risk factor in the development of these painful conditions. Finally, changes in other neurotransmitter systems within the ventral striatum, such as norepinephrine and serotonin, have been even less studied. Unfortunately, animal research addressing these questions is rare, although a decrease in opioid-stimulated dopamine release in the ventral striatum has been reported.[22] In the present study, we investigated more thoroughly changes in the ventral striatum neurotransmitters and their relationship to mechanical thresholds in animals with a neuropathic pain condition imposed by a partial injury of the sciatic nerve.

Methods

Subjects

Male C57Bl/6J (The Jackson Laboratory, Bar Harbor, ME) mice 8 to 9 weeks old at the beginning of experimentation were used. Animals were housed in groups of 4 and kept on a 12-hour light/dark cycle with food and water available ad libitum. All behavioral experimentation was performed during the light phase. Further, all procedures were conducted in accordance with the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and approved by the University of California, Los Angeles, Institutional Animal Care and Use Committee.

Surgery

Mice were randomly assigned to a neuropathic (cuff), a sham, or a naïve (no surgery) group. A total of 7 or 8 mice were included in each group. Mice undergoing surgery were anesthetized with gaseous isoflurane (induction at 5% and maintenance at 2.0–2.5% in oxygen). The lateral left thigh was shaved and disinfected with isopropyl alcohol and iodine. A 2-cm incision was made through the skin followed by a blunt dissection of the muscle to expose the sciatic nerve. Peripheral nerve ligation was performed as previously described.[20] Briefly, a 2-mm piece of PE50 tubing was opened and wrapped around the nerve using fine forceps. The skin was closed with absorbable sutures (Vicryl; Ethicon, Somerville, NJ). Sham animals received a similar surgery, but without the isolation or ligation of the nerve. After surgery, the wound was covered in antibiotic ointment and .5% bupivacaine HCl. After recovery from anesthesia, animals were returned to their home cage with food and water available ad libitum.

Mechanical Withdrawal Thresholds

Withdrawal thresholds to a mechanical stimulus applied with calibrated von Frey filaments were measured in naïve, sham, and cuff animals, as previously described.[5] Mice were placed atop a mesh grid floor in a clear acrylic glass enclosure. Von Frey filaments were applied to the plantar surface of the ipsilateral hind paw in an up-down manner, whereby filaments with increasing stiffness were applied until a paw retraction was observed. Following the first positive reaction, the next less stiff filament was applied. If no reaction was observed, the next stiffer filament was applied; if a reaction was observed, the next less stiff filament was applied. This was repeated 5 times per animal and the 50% withdrawal threshold was calculated. Baseline measurements were taken for all animals before surgery and 2 weeks postoperatively.

Neurochemical Analysis

Twenty-four hours after final behavioral testing (2 weeks after nerve injury), animals were euthanized and their brains dissected. Punches ($\sim$6 mm^3) of the ventral striatum (1.0, .445 to 1.42, $-$5.0), habenula (.5, $-$2.155 to $-$1.555, $-$2.75), anterior cingulate cortex (.0, .44 to 1.42, $-$1.5), hippocampus (1.5, $-$2.1555 to $-$1.555, $-$1.5), and amygdala (3.5, $-$2.155 to $-$1.555, $-$5.5) were isolated, flash-frozen, and stored at $-$80°C until extraction. Punches were homogenized in 300 μL of .2 M perchloric acid/.1 mM ethylenediaminetetraacetic acid with 1 μM isoproterenol as an internal standard. An aliquot was removed for protein content analysis using the Bradford assay (BioRad, Hercules, CA), and the remaining samples were centrifuged at 15,000 rpm for 10 min at 4°C. Supernatants were collected and filtered through .22-μm centrifugal filter units by spinning at 14,000 rpm for 5 minutes. The filtrate was assayed for neurotransmitter and metabolite content by high-performance liquid chromatography (HPLC) coupled to electrochemical detection. The mobile phase consisted of .1 M citrate-acetate buffer, 15% methanol, 110 mg/L sodium 1-octanesulfonate, and 5 mg/L ethylenediaminetetraacetic acid pumped at .5 mL/min through a SC-5ODS 3.0 $\times$ 150 mm column (Eicom, San Diego, CA) maintained at 25°C. The glassy carbon working electrode (WE-3G; Antec Leyden, Boston, MA) of the electrochemical detector was set at .75 V against an Ag/AgCl reference. HPLC data were collected and analyzed using EZChrom software (Agilent Technologies, Santa Clara, CA). The system was