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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.

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**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.<sup>26</sup> 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.<sup>4</sup> However, the ventral striatum is also directly involved in modulating pain itself. Lesions of striatal neurons enhance nociception and interfere with opioid analgesia.<sup>23</sup> Conversely, activation of the ventral striatum, whether by electrical stimulation or local injection of a dopamine receptor 2 agonist, produces analgesia.<sup>28,29</sup> 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 [<sup>18</sup>F]6-fluoro-L-dopa reuptake and increased dopamine receptor availability.<sup>16-18,33</sup> 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.<sup>15,16</sup> 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.<sup>25</sup> 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.<sup>12-14,31</sup> In fact, pain is the most common sensory disturbance in Parkinson's disease and is often comorbid with neuropathic pain conditions.<sup>6,24,27</sup>

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.<sup>22</sup> 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.<sup>20</sup> 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.<sup>5</sup> 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 (~6 mm<sup>3</sup>) 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-50DS 3.0 × 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

calibrated daily and sample neurochemical content normalized to the isoproterenol internal standard. Neurotransmitter content was normalized to total protein.

### Immunocytochemistry

A separate group of sham and cuff animals were sacrificed 2 weeks after nerve injury. Animals were deeply anesthetized with pentobarbital (100 mg/kg, intraperitoneal) and transcardially perfused with 4% paraformaldehyde in .1 M phosphate buffer, pH 7.4, for 5 minutes. The brain was dissected from the skull, postfixed for 30 minutes in the above-described fixative, and cryoprotected in 30% sucrose in .1 M phosphate buffer for 48 hours at 4°C.

Whole brains were flash-frozen in isopentane and embedded in an optimal cutting temperature medium (Tissue Tek OCT; Sakura Finetek Europe, Alphen aan den Rijn, The Netherlands). Forty-micrometer sections were cut at  $-20^{\circ}\text{C}$  on a cryostat (Leica, Wetzlar, Germany) and collected in phosphate-buffered saline containing .2% Triton X-100. Sections were incubated overnight with an antibody against tyrosine hydroxylase (TH; 1:1,500; EMD Millipore, Billerica, MA) at 4°C. The sections were then washed and incubated with a highly cross-adsorbed goat anti-rabbit IgG conjugated to Alexa Fluor 488 (1:400; Invitrogen, Grand Island, NY) for 2 hours at room temperature. Finally, the sections were washed in phosphate-buffered saline, mounted on slides, and cover slipped with Vectashield containing 4',6-diamidino-2-phenylindole stain (Vector Labs, Burlingame, CA).

Images were taken on a Nikon Eclipse 90i fluorescence microscope (Nikon, Tokyo, Japan) with a 20 $\times$  objective equipped with a high-resolution digital camera. For quantification, images were taken with constant exposure and gain settings. Three sections per animal were measured for each quantification criterion, and values were averaged to give a single number per animal. Cell body size was estimated by measuring the smallest possible diameter in TH-positive cells in the VTA. Only cells with a visible nucleus and strong TH labeling were measured, and between 8 and 20 cells per section were recorded. Cell counts were done on similar images with standardized area of analysis.

### Statistics

Data sets were tested for normality and variances compared using the Brown Forsythe test. When samples were normally distributed with equal variances, group means were compared using a Student's unpaired t-test or 1-way analysis of variance (ANOVA) with Tukey's multiple-comparison post hoc analysis. When samples showed unequal variance, a Mann-Whitney U test or a Kruskal-Wallis 1-way ANOVA with Dunn's multiple-comparison post hoc analysis was used to compare groups. Groups were considered statistically significant when  $P < .05$ . For neurotransmitter analysis, samples were not included in analysis if total protein concentration of the sample was below a predetermined cut-off (.1  $\mu\text{g}/\mu\text{L}$ ). Outliers, defined as data points that lie outside the range of 3 times the standard deviation of the group,

Ventral Striatal Catecholamines and Neuropathic Pain were also removed from analysis. Normalized neurotransmitter content was correlated with individual evoked mechanical thresholds using a Pearson's correlation test. Results were considered statistically significant when  $P < .05$ . Data are expressed as mean  $\pm$  standard error of the mean (SEM) throughout.

## Results

### Mechanical Thresholds

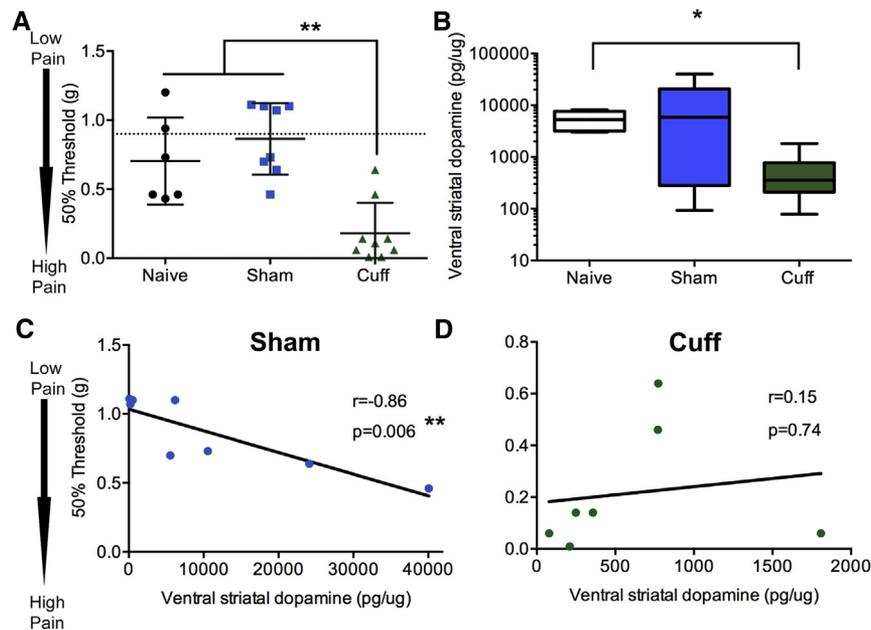
Baseline mechanical 50% thresholds as measured with von Frey filaments averaged  $.9\text{ g} \pm .06$  (Fig 1). Two weeks after baseline measurements were taken and surgery performed, mechanical thresholds were unaltered in naïve and sham groups (Fig 1A). In the cuff group, mechanical thresholds were significantly lower than in the naïve and sham groups ( $F_{(2,21)} = 15.9, P < .0001$ ).

### Dopamine Content and Correlation With Mechanical Thresholds in Control and Cuff Animals

Total dopamine content in the ventral striatum was not significantly different between naïve and sham groups, although the variability was much greater in the sham group (Fig 1B). Two weeks after nerve injury, total ventral striatal dopamine content was significantly reduced in the cuff group compared to the naïve group when compared using a Kruskal-Wallis ANOVA followed by Dunn's multiple-comparison post hoc analysis ( $K_{(2,21)} = 6.75, P = .03$ ). Dopamine content negatively correlated with mechanical thresholds in the sham group ( $r = -.86, P = .006$ ) and naïve group ( $r = -.81, P = .04$ ), with lower mechanical thresholds (higher pain sensitivity) associated with lower dopamine content in the ventral striatum (Fig 1C). This correlation between dopamine and mechanical thresholds was not evident in the cuff group (Fig 1D;  $r = .16, P = .74$ ). Dopamine content was not significantly altered in the extrastriatal brain regions, including the habenula, amygdala, and anterior cingulate cortex, but was significantly lower in the hippocampus of the cuff group (Table 1;  $U = 4, P = .04$ ). Dopamine content was not correlated with mechanical thresholds in either sham or cuff groups in any of these areas (Table 1).

### Ventral Striatal Dopamine Metabolite Content and Correlation With Mechanical Thresholds in Control and Cuff Animals

The 2 major intermediary dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT), were measured in the ventral striatum and were normally distributed with equal variances between groups. DOPAC and 3-MT content was not significantly altered between groups, although 3-MT had a tendency to be lower in the cuff group (Table 2). 3-MT and DOPAC content was not correlated with mechanical thresholds in naïve (DOPAC:  $r = -.65, P = .11$ ; 3-MT:  $r = .1, P = .87$ ), sham, or cuff groups (Table 2). The ratio of DOPAC or 3-MT to total dopamine is thought to reflect stored and released dopamine.<sup>1</sup> DOPAC/dopamine and 3-MT/dopamine ratios were not significantly altered in



**Figure 1.** Total dopamine content in the ventral striatum increased in the cuff group but lost correlation with mechanical thresholds. **(A)** Two weeks following nerve injury, mechanical thresholds of the hind paw, as measured with von Frey filaments, were significantly lowered in the cuff group. Naïve and sham groups did not have significantly different thresholds from baseline (presurgery, indicated by dotted line). Data are represented as a scatter plot. Horizontal bar represents mean, and error bars represent SEM. Groups were compared using a 1-way ANOVA followed by Tukey's post hoc analysis.  $**P < .01$ ,  $n = 6-8$ . **(B)** Dopamine content normalized to total protein in ventral striatum was measured by HPLC. Two weeks after nerve injury, cuff animals had significantly lowered dopamine content compared to the naïve group. Sham animals were not significantly different, although they had a larger variability. Data are represented as a box plot, with the horizontal line representing the mean, the outer box limits representing the interquartile span, and the whiskers the furthest data point. Groups were compared with a Kruskal-Wallis ANOVA followed by Dunn's multiple-comparison post hoc analysis.  $*P < .05$ ,  $n = 6-8$ . **(C)** Dopamine content normalized to total protein in the ventral striatum was correlated with mechanical thresholds 2 weeks after nerve injury. The sham group showed significant negative correlation between dopamine and mechanical thresholds.  $P < .01$ ,  $n = 6-8$ . **(D)** The cuff group failed to show this correlation. Correlation was performed with Pearson's correlation.  $n = 7-8$ .

any of the groups, nor were they correlated with mechanical thresholds in naïve (DOPAC/dopamine:  $r = .33$ ,  $P = .42$ ; 3-MT/dopamine:  $r = .42$ ,  $P = .48$ ), sham, or cuff groups (Table 2).

### TH-Positive Cell Labeling in the VTA

Dopaminergic cell bodies were labeled with an antibody against TH in the VTA. The number and size of TH-positive cell bodies were quantified. No difference in cell body size or number was found between the sham and cuff groups (Fig 2). No obvious morphologic

differences in cell body shape were observed between the sham and cuff groups.

### Ventral Striatal Norepinephrine Content and Correlation With Mechanical Thresholds in Control and Cuff Animals

The total ventral striatal norepinephrine content was significantly increased in the cuff group compared to naïve when compared with a Kruskal-Wallis ANOVA followed by a Dunn's multiple-comparison post hoc analysis (Fig 3A;  $K_{(2,16)} = 8.56$ ,  $P = .01$ ). In contrast to dopamine content,

**Table 1. Dopamine Content in Various Brain Regions and Correlations With Mechanical Thresholds of the Hind Paw**

	DOPAMINE CONTENT (pg/μg)				CORRELATION WITH MECHANICAL THRESHOLD			
	SHAM		CUFF		SHAM		CUFF	
	MEAN ± SEM (n)	TEST	P VALUE	R	P VALUE	R	P VALUE	
Habenula	833.0 ± 317.9 (6)	M-W	.52	-.57	.18	-.09	.86	
Amygdala	23473 ± 5172 (8)	t-test	.31	-.29	.49	.02	.96	
ACC	314.4 ± 96.5 (7)	M-W	.38	-.25	.55	.14	.79	
Hippocampus	22105 ± 5880 (8)	M-W	.04*	-.23	.59	-.29	.57	

Abbreviations: M-W, Mann-Whitney; ACC, anterior cingulate cortex.

NOTE. Dopamine levels in extrastriatal brain regions of naïve, sham, and cuff groups 2 weeks after nerve lesion. Dopamine levels were measured by HPLC and normalized to total protein of the sample. Values are expressed as group means ± SEM, followed by sample size in parentheses. Groups were compared with an unpaired Student's t-test or an M-W test. Pearson's correlation was used to compare mechanical thresholds with neurotransmitter levels.

\*Statistically significant.

**Table 2. Ventral Striatal Neurotransmitter Content and Correlations With Mechanical Thresholds of the Hind Paw**

	NEUROTRANSMITTER CONTENT (PG/ $\mu$ G)				TEST	K-WIF STATISTIC <sub>(DF)</sub> (P VALUE)	CORRELATION WITH MECHANICAL THRESHOLD			
	NAIVE	SHAM	CUFF				SHAM		CUFF	
							R	P VALUE	R	P VALUE
DA	5405.6 $\pm$ 998.1 (6)	10908 $\pm$ 5029.4 (8)	606.6 $\pm$ 225.0 (7)	K-W	6.7 <sub>(2,20)</sub> (.03)*	-.86	.006**	.16	.74	
5HT	253.3 $\pm$ 27.3 (6)	479.3 $\pm$ 63.4 (8)	394.0 $\pm$ 49.1 (8)	K-W	5.6 <sub>(2,22)</sub> (>.05)	.0004	.99	.14	.72	
NE	527.7 $\pm$ 46.2 (7)	1331.4 $\pm$ 310.5 (8)	1399.2 $\pm$ 136.6 (7)	K-W	8.6 <sub>(2,21)</sub> (.01)*	-.003	.99	.76	.04*	
DOPAC	758.2 $\pm$ 195.8 (7)	615.7 $\pm$ 190.5 (8)	551.1 $\pm$ 173.0 (8)	ANOVA	.31 <sub>(2,23)</sub> (>.05)	-.68	.58	-.06	.89	
3-MT	192.5 $\pm$ 41.1 (5)	128.2 $\pm$ 53.2 (6)	59.0 $\pm$ 22.1 (6)	ANOVA	2.56 <sub>(2,16)</sub> (>.05)	-.08	.85	-.38	.46	
DOPAC/DA	.2 $\pm$ .1 (6)	.7 $\pm$ .3 (8)	.6 $\pm$ .1 (7)	ANOVA	.19 <sub>(2,21)</sub> (>.05)	.62	.14	.76	.14	
3-MT/DA	.03 $\pm$ .002 (5)	.03 $\pm$ .01 (6)	.06 $\pm$ .02 (6)	K-W	.85 <sub>(2,16)</sub> (>.05)	-.16	.76	-.23	.66	
DA/5HT	18.5 $\pm$ 3.5 (6)	13.4 $\pm$ 5.2 (8)	2.2 $\pm$ 1.0 (7)	K-W	6.5 <sub>(2,20)</sub> (.03)*	-.92	.003**	-.01	.97	

Abbreviations: DA, dopamine; K-W, Kruskal-Wallis; 5HT, serotonin; NE, norepinephrine.

NOTE. Neurotransmitter levels in the ventral striatum of naïve, sham, and cuff groups 2 weeks after nerve lesion. Protein levels were measured by HPLC and normalized to total protein of the sample. Values are expressed as group means  $\pm$  SEM, followed by sample size in parentheses. Groups were compared with a 1-way ANOVA followed by a Tukey's multiple-comparison post hoc analysis or a Kruskal-Wallis ANOVA with a Dunn's multiple-comparison post hoc analysis. Pearson's correlation was used to compare mechanical thresholds with neurotransmitter levels.

\* $P < .05$  is statistically significant.

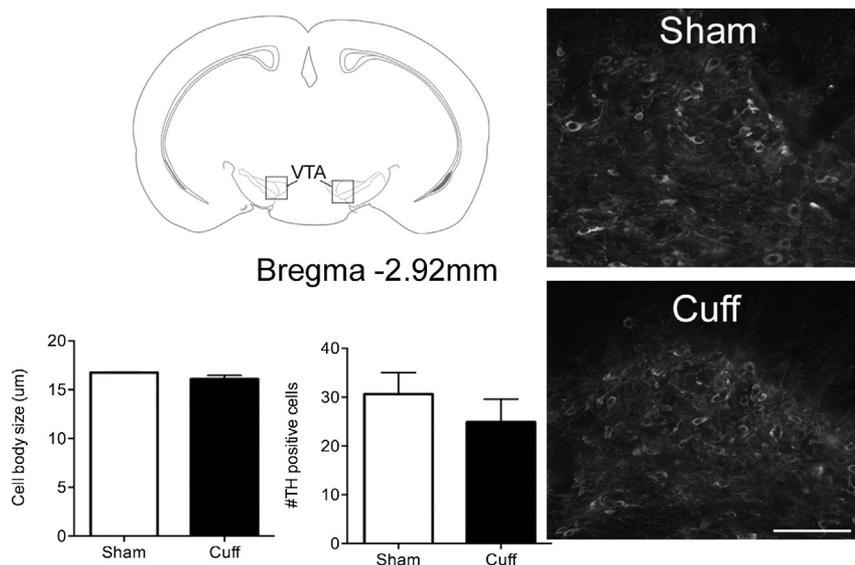
\*\* $P < .01$  is statistically significant.

norepinephrine content did not correlate with mechanical thresholds in the naïve ( $r = -.14$ ,  $P = .73$ ) or sham ( $r = -.003$ ,  $P = .99$ ) group (Fig 1C) but correlated with mechanical thresholds in the cuff group (Fig 1D;  $r = .76$ ,  $P = .04$ ).

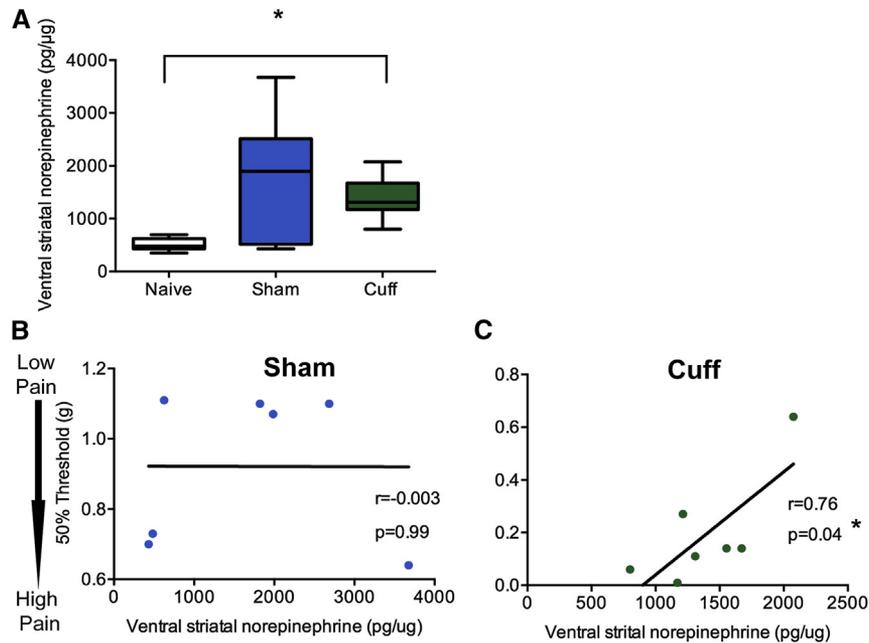
### Ventral Striatal Serotonin Content and Correlation With Mechanical Thresholds in Control and Cuff Animals

The total ventral striatal serotonin content was not significantly different among the naïve, sham, or cuff

groups, although the variability was significantly greater in the sham and cuff groups (Table 2). Serotonin content did not correlate with mechanical thresholds in the naïve ( $r = .55$ ,  $P = .15$ ), sham, or cuff group (Table 2). However, the dopamine/serotonin ratio in the ventral striatum was significantly reduced in the cuff group ( $F_{(2,21)} = 1.821$ ,  $P = .03$ ), reflecting the drop in ventral striatal dopamine in the cuff group, described in Fig 1. Further, the dopamine/serotonin ratio was negatively correlated with mechanical thresholds in the sham group ( $r = -.92$ ,  $P = .003$ ) but not the cuff group ( $r = -.32$ ,  $P = .40$ ).



**Figure 2.** No changes in the number or size of dopaminergic cells in the mesolimbic dopamine system in neuropathic pain. Tissue slices from the ventral striatum of sham and cuff groups taken 2 weeks following nerve injury were processed for immunofluorescence with an antibody against TH to label dopaminergic cell bodies in this region. Representative micrographs show TH cell bodies from sham and cuff groups. Scale bar = 100  $\mu$ m. Neither cell body size nor cell number was significantly different between sham and cuff animals. Groups were compared with an unpaired Student's t-test.  $P > .05$ ,  $n = 3-4$ .



**Figure 3.** Total norepinephrine (NE) content in the ventral striatum is significantly increased in the cuff group and highly correlated with mechanical thresholds. **(A)** NE content normalized to total protein in the ventral striatum was measured with HPLC. Two weeks after nerve injury, the cuff group had significantly elevated NE, compared to the naïve group. The sham group was not significantly different, although the variability was greater in this group. Data are represented as a box plot with the horizontal line representing the mean, the outer box limits representing the interquartile span, and the whiskers the furthest data point. Groups were compared with a Kruskal-Wallis ANOVA followed by Dunn's multiple-comparison post hoc analysis.  $*P < .05$ ,  $n = 7-8$ . NE content normalized to total protein in the ventral striatum were not correlated with mechanical thresholds in **(B)** sham animals but were highly correlated in **(C)** cuff animals 2 weeks after nerve injury. Correlation was performed with Pearson's correlation.  $*P < .05$ ,  $n = 7$ .

## Discussion

The present study describes significant changes in neurotransmitter content in the ventral striatum of neuropathic pain (cuff) animals. A decrease in ventral striatal dopamine with a concomitant rise in norepinephrine content was associated with a decrease in mechanical withdrawal thresholds indicative of neuropathic pain. Mechanical thresholds shifted from being correlated with dopamine to being correlated with norepinephrine in neuropathic pain, which suggests adaptations in ventral striatal neurochemistry that may underlie the pathologic pain associated with nerve injury.

A relationship between striatal dopamine and pain thresholds in healthy and neuropathic pain states has been established. Human imaging PET studies found increased dopamine receptor availability in the striatum that was positively correlated to pain thresholds.<sup>32</sup> In other words, increased binding of the labeled dopamine agonist was associated with less subjective pain. The findings in this study support these studies, where low ventral striatum dopamine content was associated with high mechanical thresholds in the naïve and sham groups. This suggests that occupied dopamine receptors, and, therefore, endogenous dopamine that would occupy those receptors, are related to the pain threshold of the healthy individual. Importantly, much like the results we observed in mice, the relationship between striatal dopamine and pain thresholds was lost in fibromyalgia patients.<sup>33</sup> However, the conclusions from these PET imaging studies have been limited because they cannot address whether changes in dopamine receptor

occupancy are due to changes in receptor number, PET ligand affinity/accessibility, or occupation by endogenous dopamine. Our study supports the human imaging finding that striatal dopamine signaling is disrupted in neuropathic pain, and it extends these results by showing that changes in dopamine receptor occupancy in the striatum may be due, at least in part, to decreased endogenous dopamine content. An important limitation to this study is the inability to distinguish between intracellular and released dopamine. Although overall reductions in total dopamine content within the striatum suggest lowered dopamine function, further studies are needed to explore changes in *in vivo* dopamine release. Encouragingly, an initial study in this area suggests that dopamine release is, in fact, decreased in chronic pain animals.<sup>22</sup>

The high variability in dopamine content in the striatum of sham animals is surprising given that naïve (non-surgery) animals do not show this same variability. This suggests that sham surgery alone is able to induce some neurochemical changes in the brain and may represent an important endogenous analgesic response of the animal. This is supported by our observation that striatal dopamine content was significantly correlated with mechanical pain thresholds in sham animals. There is considerable animal research to support the idea that striatal dopamine contributes to analgesia. Electrical stimulation of the VTA produced analgesia, and lesions of the striatum and VTA increased pain sensitivity.<sup>23,28</sup> It should be noted that our sample size is relatively small in this study, and some of the variability could be due to sampling error. Although these results are supported

by previous literature, more studies replicating these findings are needed to further validate these results.

Further, although the sham group had variable dopamine content, the levels were not significantly different from those of the naïve group. This is in contrast to the cuff group, which showed significantly reduced ventral striatal dopamine contents (Fig 1B). Depletion of monoamines in the mesolimbic dopamine pathways can lead to a hypersensitive pain state.<sup>21</sup> Our results support these studies by showing that the depletion of dopamine in neuropathic pain is related to lowered mechanical thresholds, albeit not correlated with mechanical thresholds (Fig 1D). The lack of correlation with mechanical thresholds could be due to the fact that the spread of mechanical thresholds is restricted in cuff animals, given that they fall close to the lower limit of the testing threshold. We also measured changes in dopamine content in other brain regions that receive strong or moderate input from the VTA, including the habenula, anterior cingulate cortex, hippocampus, and amygdala (Table 1). Dopamine content in the habenula, amygdala, or anterior cingulate cortex did not differ significantly between the naïve, sham, and cuff groups. Although dopamine content was significantly lower in the hippocampus of the cuff group, it was never correlated with mechanical thresholds in either the sham or cuff group in any of these brain regions. Therefore, the decreased dopamine content observed in neuropathic pain was not reflective of a global loss of dopamine content throughout the brain, but rather highlighted ventral striatum dopamine as being particularly affected by this chronic pain condition. The decreased dopamine in the hippocampus of the cuff group is striking and may contribute to other elements of the chronic pain state beyond evoked pain thresholds.

In addition to dopamine, we analyzed changes in dopamine metabolites in the ventral striatum (Table 2). The 2 major intermediary metabolites of dopamine, DOPAC and 3-MT, are formed from separate metabolic pathways of separate pools of dopamine, with DOPAC formed intracellularly and 3-MT formed in the extracellular space. Therefore, DOPAC and 3-MT content can reflect stored versus released dopamine content, particularly when expressed as an index of total dopamine content.<sup>1</sup> Neither absolute DOPAC content nor DOPAC/dopamine ratios were significantly different among the naïve, sham, and cuff groups, suggesting that there was no change in the stored dopamine at the terminals. Although neither 3-MT content nor 3-MT/dopamine ratios were significantly different, 3-MT content tended to be lower in the cuff group. This suggests a decrease in released dopamine, which is supported by previous research that found decreased morphine-stimulated dopamine release in neuropathic animals.<sup>22</sup> However, neither DOPAC nor 3-MT content (nor DOPAC/dopamine or 3-MT/dopamine ratios) correlated with mechanical thresholds in the naïve, sham, or neuropathic group, suggesting that dopamine metabolites contribute less to mechanical thresholds in healthy or neuropathic pain states than does the total dopamine content.

The loss of dopamine in the cuff group is significant, which may result from a loss of dopaminergic cells within

the mesolimbic dopamine system. To test this hypothesis, we performed immunocytochemical staining against TH, a marker of dopaminergic neurons. No significant difference in cell body size or number between sham and cuff groups was detected. Therefore, loss of dopamine in the ventral striatum must be due to the overall dopamine content in the terminals rather than dopaminergic cell death.

In contrast to dopamine, norepinephrine content was significantly elevated in the ventral striatum of the cuff group compared to naïve controls (Fig 3A). Although norepinephrine content did not correlate with mechanical thresholds in the sham group, it showed a strong positive correlation with mechanical thresholds in the cuff group. Although the contribution of ventral striatal norepinephrine to pain states has not been well described, in general, norepinephrine is involved in attention, general arousal, and stress reactions in challenging environments.<sup>2,3</sup> Increased norepinephrine in the striatum of cuff animals could lead to increased arousal following a painful stimulus that could translate into a lowered mechanical threshold. Further research in this area is warranted to describe the contribution of norepinephrine in the ventral striatum to pain sensitivity.

Serotonin content was also measured in the ventral striatum of naïve, sham, and neuropathic groups and did not differ significantly, although it tended to be higher in the cuff group. However, serotonin content in the ventral striatum did not correlate with mechanical thresholds in either the sham or cuff group (Table 2). This was surprising given that the ventral striatum receives strong input from the dorsal raphe and encodes mainly aversive information, which would be particularly relevant in painful conditions.<sup>7,8</sup> However, the ratio of dopamine to serotonin was significantly reduced in the cuff group, reflecting the above-described drop in dopamine content and rise in serotonin, and this ratio was correlated with mechanical thresholds in the cuff group but not the sham group. Previous studies have found that dopamine and serotonin interact in an opposing manner in the striatum, with dopamine mediating reward and serotonin mediating aversion.<sup>10,11,19</sup> It has been proposed that it is the balance of serotonin and dopamine that refines the behavioral response to motivationally relevant stimuli by balancing approach and withdrawal.<sup>9</sup> Our results support this proposition given the tight correlation of the dopamine/serotonin ratio with mechanical thresholds in sham animals. Previous studies have also found the dopamine/serotonin ratio to be correlated with the degree of naloxone place aversion.<sup>30</sup> The implication of the dopamine/serotonin ratio in mediating evoked pain thresholds remains to be described, although our results suggest that it may play a particularly important role.

Overall, these results show a striking change in the ventral striatal catecholamine content in neuropathic pain states, with a shift in pain thresholds that are correlated with dopamine to norepinephrine systems. Additional research is warranted to further explore the contribution of norepinephrine in the striatum to mediate neuropathic pain.

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