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Brain-derived neurotrophic factor in the mesolimbic reward circuitry mediates nociception in chronic neuropathic pain

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Abstract

BACKGROUND—The mesolimbic reward system plays a critical role in modulating nociception; however, its underlying molecular, cellular, and neural circuitry mechanisms remain unknown.

METHODS—Chronic constrictive injury (CCI) of the sciatic nerve was used to model neuropathic pain. Projection-specific *in vitro* recordings in mouse brain slices and *in vivo* recordings from anesthetized animals were used to measure firing of dopaminergic (DA) neurons in the ventral tegmental area (VTA). The role of VTA–nucleus accumbens (NAc) circuitry in nociceptive regulation was assessed using optogenetic and pharmacological manipulations, and the

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underlying molecular mechanisms were investigated by Western blotting, enzyme-linked immunosorbent assays, and conditional knockdown techniques.

RESULTS—c-Fos expression in and firing of contralateral VTA–NAc DA neurons were elevated in CCI mice, and optogenetic inhibition of these neurons reversed CCI-induced thermal hyperalgesia. CCI increased the expression of brain-derived neurotrophic factor (BDNF) protein but not mRNA in the contralateral NAc. This increase was reversed by pharmacological inhibition of VTA DA neuron activity, which induced an antinociceptive effect that was neutralized by injecting exogenous BDNF into the NAc. Moreover, inhibition of BDNF synthesis in the VTA with anisomycin or selective knockdown of BDNF in the VTA–NAc pathway were antinociceptive in CCI mice.

CONCLUSIONS—These results reveal a novel mechanism of nociceptive modulation in the mesolimbic reward circuitry and provide new insight into the neural circuits involved in the processing of nociceptive information.

Keywords

ventral tegmental area; nucleus accumbens; mesolimbic reward system; dopaminergic neuron; BDNF; nociception; pain

Brain imaging studies using preclinical models and human patients have shown that the mesolimbic reward circuitry, which is composed of dopaminergic (DA) neurons of the ventral tegmental area (VTA) and their projections to several brain regions, is critical for pain sensation and pain-related emotional experiences (1–8). Functional magnetic resonance imaging in healthy subjects revealed activation of the reward circuitry by noxious stimuli (8–10). Animal studies also showed that DA neurons in the VTA are activated by multiple nociceptive stimuli, such as electrical foot shock, formalin, and chronic nerve injury (2, 4, 8). Activated VTA DA neurons modulate pain through their projection regions (6,9–11).

An increasing number of studies have investigated the role of pathological adaptations of medium spiny neurons in the nucleus accumbens (NAc), a main target of VTA DA neurons, in nociceptive modulation and pain-related affective behaviors (10–15). Several early studies found that the activation of DA neurons, arising in the VTA and projecting to the NAc, plays an important role in suppressing tonic pain (16–18). Furthermore, this system was also found to be involved in the pain relief effects of opioid and psychostimulant drugs and in the pain- or stress-induced inhibition of nociception via the release of endogenous opioids and substance P in the midbrain (19–22). Later studies revealed a different role of the mesolimbic DA system in modulating nociception. For instance, functional imaging studies of human and rat brains indicate positive neuronal activation in the NAc in response to noxious stimuli (10, 23, 24). An animal study found that the blockade of NAc activation attenuated hyperalgesia and other pain-like behaviors in a noxious model of jaw opening (4). The NAc regulates descending pain modulatory pathways and may also modulate directly or indirectly the perception of noxious information (4, 6, 10, 23, 25, 26). On the contrary, analgesia/pain relief was reported to activate VTA DA neurons and enhance DA release in the NAc (27). These studies support possible and diverse roles of the mesolimbic reward

circuit in nociceptive modulation. A better understanding of the molecular and cellular mechanisms of nociceptive modulation in this emotion-related circuitry is needed.

Although the mesolimbic reward system is involved in multiple aspects of pain processing, in this study, we mainly investigated how VTA–NAc circuitry modulates thermal hypersensitivity under neuropathic pain states. Brain-derived neurotrophic factor (BDNF) signaling within the mesolimbic reward system has been well investigated in a variety of adaptive and pathological behaviors (28–33). Phasic optogenetic activation of VTA–NAc DA neurons increases BDNF levels in the NAc in socially stressed mice (34). Such DA neuron activation and increases in NAc BDNF signaling also promote a susceptibility to social defeat stress, whereas decreases in BDNF levels in this VTA–NAc pathway reduce the susceptibility to deleterious effects of stress and induce antidepressant-like responses (28, 29). Based on these findings, we hypothesize that BDNF signaling in the VTA–NAc pathway is involved in modulating nociception in chronic pain. Here, we reveal a BDNF-mediated modulatory mechanism within the VTA–NAc circuitry in a chronic neuropathic pain model.

METHODS AND MATERIALS

Animals

Male 7–8 week-old C57BL/6J, *Bdnf^{loxP/loxP}* (B6129PF2/J background), and B6129PF2/J mice were housed at 22–25°C with a 12-h light-dark cycle and were fed *ad libitum*.

Sciatic nerve chronic constrictive injury (CCI) model

CCI was performed according to the method of Bennett and Xie (35). In brief, mice (~9 weeks old) were anesthetized and the left sciatic nerves were exposed at the mid-thigh level. The constriction injuries were performed proximal to the trifurcations with three loose ligatures using a 5-0 silk thread at 1-mm intervals.

Behavioral testing

Thermal nociception was assessed by measuring paw withdrawal latencies (PWLs) in response to thermal stimulation according to the method described by Hargreaves *et al.* (36, 37). Behavioral testing was carried out in a blinded manner. Detailed timelines for each experiment are presented in Figures 1A, 2A, 3A, 4E, 5A, and 6A.

Stereotaxic surgery and microinjection

Animals were anesthetized and positioned in a small-animal stereotaxic instrument (David Kopf Instruments, Tujunga, CA). A Hamilton syringe needle (33-gauge) was used to unilaterally infuse Lumafluor, virus, or chemicals in a volume of 0.15–0.5 μ L at a rate of 0.1 μ L/min with a microinfusion pump (Harvard Apparatus, Holliston, MA).

Optogenetic techniques

Optogenetic silencing experiment in NAc-projecting VTA neurons was performed as described in our previous study (38). For all behavioral experiments, mice were given 8-s-on/2-s-off stimulation during thermal nociception testing using unilateral stimulations.

***In vitro* and *in vivo* electrophysiology**

Cell-attached recordings in acute brain slices and *in vivo* recordings from anesthetized animals were used to measure the firing rates of VTA DA neurons according to previous reports (38–40).

Statistical analyses

Data were analyzed with PRISM software (GraphPad) and are expressed as means $\pm$ standard errors. One-way analyses of variance (ANOVAs) were used to compare results of behavioral and recording data from three or more groups followed by post hoc Newman–Keuls multiple comparisons tests. A two-way ANOVA was used to analyze the behavioral data from different time points, with a post hoc Newman–Keuls multiple comparisons test, when appropriate. Student's *t* tests were used for analyses between two groups. Main and interaction effects were considered significant at $p < 0.05$.

RESULTS

CCI increases c-Fos expression in VTA DA neurons

We first studied VTA neuron activity in CCI mice, which reliably induces a neuropathic pain-like behavioral response (35). CCI mice exhibited a significant decrement in thermal PWLs in the affected hind paws, while this decrement was absent in the contralateral hind paws and in sham-treated animals (two-way ANOVA, $F_{3,178}=4.08$, $p < 0.0001$; Fig. S1B). On day 7 after CCI surgery, we measured the expression of c-Fos protein, a molecular marker of neuronal activation, in the VTA and found that CCI induced a significant increase in the VTA contralateral to the CCI compared with the sham group and the ipsilateral VTA (one-way ANOVA, $F_{3,20}=108.2$, $p < 0.0001$; Fig. S1C,D). Few c-Fos⁺ neurons were detected in the substantia nigra, another DA neuron-rich brain region in close proximity to the VTA (Fig. S1C). Double immunofluorescence staining for tyrosine hydroxylase (TH, regularly used as a marker for dopaminergic neurons) further confirmed that the number of c-Fos⁺/TH⁺ cells (Fig. S1E; unpaired *t* test, $t_{6}=6.853$, $p < 0.001$; Fig. S1F) and the percentages of c-Fos⁺/TH⁺ neurons within the populations of TH⁺ (unpaired *t* test, $t_{6}=3.719$, $p < 0.01$; Fig. S1E,G) and c-Fos⁺ (unpaired *t* test, $t_{6}=3.422$, $p < 0.05$; Fig. S1E,H) positive neurons in the VTA were increased in the CCI group.

CCI increases overall firing rates and bursting firing patterns of VTA DA neurons

To further determine the changes in neuronal activity of VTA DA neurons in the chronic neuropathic pain state, we performed *in vitro* recordings in acute VTA slices from CCI mice and sham controls (Fig. 1A). We found that CCI caused a 51% increase in firing rates in contralateral, but not ipsilateral, VTA DA neurons (Fig. 1B,C,D) (one-way ANOVA, $F_{3,144}=10.35$, $p < 0.0001$; Fig. 1C). Moreover, there was a significant inverse correlation between the average VTA firing rate for each animal and its PWL (Fig. 1E). It is known that VTA DA neurons exhibit two types of firing patterns *in vivo*: single-spike tonic firing and high-frequency bursting firing. Bursting firing by VTA DA neurons transiently increases the release of DA and BDNF onto the postsynaptic neurons (34, 41). However, this important bursting firing pattern is absent in VTA slice preparations (42). Therefore, we utilized *in*

vivo single-unit extracellular recordings from anesthetized mice to study the firing rates and firing patterns in VTA DA neurons. Consistent with the *in vitro* data, we found that CCI induced a 42% increase in the overall firing activity in contralateral, but not in ipsilateral, VTA DA neurons (Fig. 1F,G,H) (one-way ANOVA, $F_{3,137}=5.106$, $p < 0.001$; Fig. 1G, left). Importantly, the percentage of spikes within bursts in contralateral VTA DA neurons was dramatically increased compared with the ipsilateral hemisphere in CCI mice or sham controls (one-way ANOVA, $F_{3,68}=7.007$, $p < 0.001$; Fig. 1G, right). Consistently, the *in vivo* firing rates and the percentages of spikes within bursts for each group were significantly correlated with thermal pain latencies (Fig. 1I). These electrophysiological results suggest that changes in the overall firing rates and bursting firing patterns of VTA DA neurons play an important role in mediating the nociceptive response.

Inhibition of VTA DA neuron firing reverses thermal hyperalgesia in CCI mice

To explore the functional relationship of thermal nociceptive responses and VTA DA neuron activity, we assessed whether pharmacological inhibition of VTA DA neuron activity regulates the established thermal hyperalgesia in CCI mice. It is well known that VTA DA neuron activity is regulated by intrinsic ion channels, such as the hyperpolarization-activated cation channel (I_h), and extrinsic synaptic inputs, including those from γ -aminobutyric acid (GABA)-ergic neurons (39, 43, 44). Here, we pharmacologically inhibited VTA DA neuron activity with I_h blockers (ZD7288, 0.1 $\mu\text{g}/0.15 \mu\text{L}$ or DK-AH 269, 0.6 $\mu\text{g}/0.15 \mu\text{L}$) or the selective GABA_B receptor agonist, baclofen (0.1 $\mu\text{g}/0.15 \mu\text{L}$), the reagents that are known to reliably decrease VTA DA neuron firing (39–44). ZD7288, DK-AH 269, or baclofen was microinfused into the VTA 30 min before behavioral testing on day 7 after CCI surgery (Fig. 2A,B). Behavioral results showed that ZD7288 (two-way ANOVA, main effect group: $F_{4,186}=89.51$, $p < 0.0001$; main effect time: $F_{5,186}=24.07$, $p < 0.0001$; group $\times$ time interaction: $F_{20,186}=5.60$, $p < 0.0001$; Fig. 2C) and baclofen (two-way ANOVA, main effect group: $F_{4,180}=116.82$, $p < 0.0001$; main effect time: $F_{5,180}=34.44$, $p < 0.0001$; group $\times$ time interaction: $F_{20,180}=7.32$, $p < 0.0001$; Fig. 2E) had a 2-h analgesic effect, while DK-AH 269 (two-way ANOVA, main effect group: $F_{4,267}=184.92$, $p < 0.0001$; main effect time: $F_{8,267}=22.30$, $p < 0.0001$; group $\times$ time interaction: $F_{32,267}=6.39$, $p < 0.0001$; Fig. 2D) displayed a long-lasting effect (~ 72 h). These effects were not seen when the drugs were infused into the substantia nigra (Fig. 2B,C,D,E). In additional control experiments, infusion of these drugs into the VTA did not induce observable changes in motor function (Fig. S2). These findings suggest that the increased activity of DA neurons within the contralateral VTA contributed to the maintenance of CCI nociceptive responses and to pain sensation modulation.

CCI activates contralateral VTA–NAc-projecting DA neurons

The NAc is an important brain region downstream of the VTA, and structural and functional plasticity in the NAc have been associated with chronic pain (12, 13, 23, 45–48). Thus, we investigated the functional role of VTA–NAc circuitry in modulating neuropathic pain. To do this, we injected a retrograde tracer, Lumafluor, into the NAc 7 days before CCI. We then performed double immunofluorescence staining for c-Fos and the retrograde tracer in the VTA 7 days after CCI (Fig. 3A). Our results show that, in NAc-projecting VTA neurons, the total numbers (unpaired *t* test, $t_6=8.460$, $p < 0.0001$; Fig. 3B and C, left) and percentages

(unpaired t test, $t_{6}=6.627$, $p < 0.001$; Fig. 3B and C, middle) of c-Fos-expressing neurons increased in CCI mice. Additionally, higher proportions ($>60\%$) of c-Fos⁺ VTA neurons projected to the NAc in CCI mice (unpaired t test, $t_{14}=3.253$, $p < 0.05$; Fig. 3B and C, right). We also monitored the firing rates of these retrograde tracer-labeled VTA-to-NAc projecting neurons in VTA slices from CCI mice or sham controls (Fig. 3A,D). Consistent with our data recorded from the overall population of VTA DA neurons, recordings from retrogradely labeled neurons in the VTA contralateral to CCI revealed a significantly higher firing rate as compared with sham mice (unpaired t test, $t_{8}=3.839$, $p < 0.01$; Fig. 3E), and this increased firing was accompanied by an increase in the I_h current (two-way ANOVA, $F_{1,140}=45.15$, $p < 0.0001$; Fig. 3F). To further confirm the findings on firing activity, we measured c-Fos protein expression in the NAc of control mice and those with CCI. Our results show that CCI significantly increased the expression of c-Fos protein in the NAc contralateral to CCI, and interestingly, this increase was partially reversed by pharmacological inhibition of VTA DA neuron firing through intra-VTA microinjection of DK-AH 269 or baclofen (one-way ANOVA, $F_{4,16}=26.39$, $p < 0.0001$; Fig. 3G).

Optogenetic inhibition of VTA neurons projecting to NAc reverses thermal hyperalgesia in CCI mice

To further investigate the causal relationship between the activity of VTA–NAc projections and nociceptive responses, we injected a retrograde-traveling adeno-associated virus expressing Cre recombinase (AAV2/5-Cre) into the NAc, and an AAV vector expressing Cre-dependent halorhodopsin-3.0 (AAV-DIO-NpHR3.0-eYFP) was injected into the VTA to express NpHR3.0 selectively in NAc-projecting VTA neurons (Fig. 4A). TH/eYFP double-immunofluorescence staining validated viral expression, and quantification showed that 97.8% of eYFP⁺ neurons were TH⁺ (Fig. 4B). Using whole-cell current-clamp recording, we confirmed that the firing of VTA–NAc neurons was reliably inhibited by optical stimulation (8 s on/2 s off) (Fig. 4C) using a stimulation pattern that is known not to induce rebound (38). Our behavioral results show that *in vivo* optical inhibition of VTA neurons projecting to the NAc reversed the established thermal hyperalgesia in affected hind paws of CCI mice, whereas this effect was not observed in sham-treated mice (two-way ANOVA, main effect group: $F_{2,34}=32.24$, $p < 0.0001$; main effect time: $F_{1,34}=4.98$, $p = 0.0323$; group $\times$ time interaction: $F_{2,34}=5.64$, $p = 0.0077$; Fig. 4D,E,F). These data demonstrate that the increased activity of contralateral VTA–NAc-projecting neurons is necessary for maintaining the thermal hyperalgesia induced by CCI.

BDNF signaling in mesolimbic reward circuitry modulates nociception in CCI mice

BDNF signaling plays a well-established role in modulating the excitation of the mesolimbic reward system in psychiatric disorders (25, 28, 31, 33, 49). Thus, we investigated the potential role of BDNF signaling within the VTA–NAc circuitry in modulating the nociception that underlies chronic neuropathic pain. To do this, we examined the pathway-specific expression of *Bdnf* in VTA-to-NAc-projecting cells. Fluorescent *in situ* hybridization revealed an upregulation of *Bdnf* mRNA in NAc-projecting VTA neurons of CCI mice (Fig. 5A,B). Next, we dissected contralateral VTA and NAc tissues to measure mRNA and protein levels of BDNF in CCI mice and sham controls (Fig. 5A). The results show significantly increased levels of *Bdnf* mRNA in the VTA but not in the NAc (VTA:

unpaired t test, $t_{6}=4.825$, $p = 0.0029$; NAc: unpaired t test, $t_{6}=0.2387$, $p = 0.8193$; Fig. 5C) and significantly increased levels of BDNF protein in the VTA and the NAc (VTA: unpaired t test, $t_{10}=3.099$, $p = 0.0092$; NAc: unpaired t test, $t_{10}=2.727$, $p = 0.0184$; Fig. 5D), as compared with the sham controls. Microinjections of DK-AH 269 or baclofen into the VTA to inhibit VTA DA neuron firing reversed the CCI-induced increase in BDNF protein in the NAc compared with vehicle-injected controls (VTA: unpaired t test, $t_{8}=2.836$, $p = 0.0210$; NAc: unpaired t test, $t_{8}=3.187$, $p = 0.0129$; Fig. 5E). Moreover, we found that the basal release of BDNF in the NAc was significantly increased in CCI mice compared with that in sham controls, and this was inhibited by intra-VTA injection of DK-AH 269 (one-way ANOVA, $F_{2,6}=77.51$, $p < 0.0001$; Fig. 5F). Furthermore, intra-NAc microinjection of TrkB-Fc (20 ng/0.15 μ l), a BDNF scavenger that blocks BDNF/TrkB signaling, produced a significant analgesic effect in CCI mice compared to the effect from control IgG-Fc, and this effect lasted for at least 2 h (two-way ANOVA, main effect group: $F_{3,156}=85.82$, $p < 0.0001$; main effect time: $F_{5,156}=11.99$, $p < 0.0001$; group $\times$ time interaction: $F_{15,156}=5.03$, $p < 0.0001$; Fig. 5G). Moreover, the antinociceptive effects induced by intra-VTA injection of baclofen (two-way ANOVA, main effect group: $F_{1,130}=10.01$, $p = 0.0019$; main effect time: $F_{6,130}=16.59$, $p < 0.0001$; group $\times$ time interaction: $F_{6,130}=3.46$, $p = 0.0033$; Fig. 5H) and DK-AH 269 (two-way ANOVA, main effect group: $F_{1,160}=11.59$, $p = 0.0014$; main effect time: $F_{9,160}=24.69$, $p < 0.0001$; group $\times$ time interaction: $F_{9,160}=2.76$, $p = 0.0050$; Fig. 5I) in CCI mice were prevented by prior microinjections of exogenous BDNF (10 ng/0.15 μ l) into the NAc.

Pathway-specific BDNF signaling in VTA–NAc circuitry modulates nociception in CCI mice

Under physiological and control conditions, *Bdnf* is transcribed at very low levels in NAc neurons, and it is known that the VTA is the major source of BDNF protein in the NAc (25, 28). Therefore, we hypothesized that enhanced release of BDNF from activated VTA DA neurons in the NAc modulates the nociceptive responses. To test this possibility, we examined thermal hypersensitivity in CCI mice after microinjecting a protein synthesis inhibitor (anisomycin, 20 μ g/0.15 μ L) into the VTA to block nonspecifically BDNF synthesis (Fig. 6A). The results show that treatment with anisomycin, but not its vehicle, attenuated CCI-induced thermal hyperalgesia (two-way ANOVA, main effect group: $F_{3,126}=119.16$, $p < 0.0001$; main effect time: $F_{5,126}=14.81$, $p < 0.0001$; group $\times$ time interaction: $F_{15,126}=7.36$, $p < 0.0001$; Fig. 6B), which was reversed by intra-NAc injections of exogenous BDNF (10 ng/0.15 μ L) (two-way ANOVA, main effect group: $F_{1,66}=5.27$, $p = 0.0249$; main effect time: $F_{5,66}=44.94$, $p < 0.0001$; group $\times$ time interaction: $F_{5,66}=4.55$, $p = 0.0013$; Fig. 6C), indicating that protein synthesis in the VTA–NAc circuitry contributes to the modulation of chronic pain-related thermal hypersensitivity. To further test the causal relationship between nociceptive response modulation and BDNF signaling in the VTA–NAc circuitry, we injected AAV2/5-Cre into the NAc of *Bdnf^{loxP/loxP}* mice (Fig. 6D) to selectively knockdown BDNF expression in NAc-projecting VTA neurons (Fig. 6E,F), and we found a significant reversal of CCI-induced thermal hyperalgesia (one-way ANOVA, $F_{2,19}=6.866$, $p < 0.01$; Fig. 6G). Collectively, these data suggest that the release of BDNF in the NAc results from activity of VTA–NAc circuitry, and this is sufficient to modulate thermal pain behavior under chronic neuropathic pain conditions.

DISCUSSION

Accumulating evidences suggest that midbrain DA neurons modulate acute and chronic pain, as well as the process of analgesia/pain relief (1–8,27). However, the large divergence in results from various studies demonstrates the need to detail the underlying mechanisms in the midbrain DA system regulating pain and analgesia. We found that CCI-induced chronic neuropathic pain increased tonic activity and burst firing in contralateral VTA DA neurons and that pharmacological inhibition of this activity attenuated established thermal hyperalgesia. These findings suggest that VTA DA neuron activation plays an important role in mediating chronic pain and are consistent with those from a recent study using another widely used animal model of neuropathic pain, mice with spared nerve injury (14). *In vivo* electrophysiological recordings from those mice revealed an increase in phasic firing activity in the contralateral VTA DA neurons and enhanced DA release in the NAc. In another study, fast-scan cyclic voltammetry demonstrated that a painful tail pinch elicits DA release in the core region of the NAc in anesthetized rats (50).

By contrast, the traditional notion, and one that the majority of clinical and animal studies support is that analgesia/pain relief is a type of reward and leads to midbrain DA reward system activation. *Ex vivo* electrophysiological recordings from mice with spared nerve injuries showed decreased tonic firing in medial VTA DA neurons (50). The release of DA in the shell region of the NAc is also increased after peripheral nerve block in rats with incisional pain (27). Importantly, midbrain DA neurons are anatomically and functionally highly heterogeneous, which may account for these conflicting findings, as well as those in other research fields, including those studying depression (38, 51, 52). Though not shown in this report, we observed a decreased firing activity of medial VTA neurons that project to the medial prefrontal cortex. This provides additional support for divergent results from different projections within mesolimbic circuits. Additionally, conflicting results may be obtained with different animal models.

The NAc, which is a primary target of VTA projections, has been implicated in mediating responses to noxious stimuli in human functional brain imaging studies (10–11). Animal studies have also indicated that inhibiting NAc activation attenuates pain behavior, while activating NAc neurons enhances pain behavior, in a noxious jaw-opening reflex model (1, 5). From these studies, a link has been made between the modulation of neuronal activity in the NAc and pain modulation. However, NAc-related neural circuitry mechanisms underlying pain states, especially chronic neuropathic pain, are poorly understood. In this study, we found that CCI surgery increased tonic firing of NAc-projecting VTA neurons, and optogenetic inhibition of these neurons during behavioral testing significantly increased PWLs to thermal stimulation (analgesic effect). VTA-to-NAc-projecting DA neurons express larger I_h currents, which positively correlate with neuronal firing activity. We observed that pharmacologically inhibiting VTA DA neuron firing with I_h blockers reversed CCI-induced NAc neuron activation and thermal hyperalgesia in CCI mice. These data further support the role of VTA–NAc pathways in modulating CCI-induced thermal hyperalgesia. The VTA comprises a heterogeneous population of DA-, GABA-, and glutamate-releasing neurons; DA neurons are the most abundant, while GABA and glutamate neurons account for ~30% and 2–5% of the total population, respectively. VTA GABA neurons projecting to the NAc

represent ~25% of all VTA GABA cells (55), which corresponds to ~7–8% of the total cell population in the VTA. Our previous work using retrograde Lumafluor and virus (two widely used retrograde-labeling approaches) showed that ~95–98% of VTA-to-NAc-projecting cells are DA neurons (38). Three decades ago, a retrolabeling study using True blue indicated that 85% of VTA projections to the NAc are dopaminergic (53). The difference between the results of that study and ours might result from the difference in approaches. Altogether, most VTA neurons that project to the NAc are DA neurons, and these neurons are largely responsible for the effects we observed. Nonetheless, we cannot exclude contributions that may come from a relatively small population of projecting GABA neurons, which were recently reported to be sufficient to mediate learning behavior (54).

Multiple bodies of evidence have demonstrated that spinal BDNF signaling modulates nociceptive transmission and central sensitization under chronic pain states (55–57). However, less information is available at the supraspinal level, especially in the mesolimbic reward circuitry. As previously mentioned, BDNF signaling within the mesolimbic reward system has been implicated in several neuropsychological disorders (15, 25, 28, 31–33, 55, 58). Here, we found that 1) CCI-induced BDNF protein expression and release in the NAc depended on VTA DA neuron activity; 2) the inhibition of VTA DA neuron firing reversed the established thermal hyperalgesia in CCI mice, which was restored by microinjecting exogenous BDNF into the NAc; 3) the blockade of BDNF signaling in the NAc and inhibition of synthesis in the VTA produced analgesia effects, which were reversed by injecting BDNF directly into the NAc; and 4) selective knockdown of BDNF expression in NAc-projecting VTA neurons reversed CCI-induced thermal hyperalgesia. Previous studies have demonstrated that anterograde axonal BDNF transport occurs within this circuit (59, 60), and there is a strong association between VTA firing rates and BDNF levels in the NAc target region (34). Together with previous findings, this study provides comprehensive evidence that mesolimbic BDNF signaling is a critical nociceptive effector.

In this study, all of the electrophysiological recordings, c-Fos immunohistochemistry, and changes in BDNF protein levels were performed from animals that did not receive thermal stimulation. We believe that evoked pain and pain from resting thermal hyperalgesia engage different mechanisms. On the basis of currently available studies and our present data, BDNF signaling is important under a hyperalgesic state (55–57). Functional MRI might be a suitable approach to measure brain activity in response to imposed thermal stimulation. A clinical study using positron emission tomography with a specific radiotracer in human subjects found increased DA D2 receptor activation after painful stimulation (61). In future studies, these functional imaging techniques may be able to measure real-time brain activity and molecular signaling activation during thermal stimulation. However, there are no studies currently investigating the role of BDNF signaling in thermal stimulation-evoked pain behaviors under hyperalgesic states, and thus it is difficult to predict the role of BDNF in this process.

In conclusion, our findings consistently demonstrate that long-lasting nociceptive input activates VTA neurons and, via increased release of BDNF, induces nociception-coded actions in NAc neurons, thereby promoting behavioral thermal hypersensitivity. Thus, this study reveals a novel mechanism for BDNF-mediated modulation of nociception in the

mesolimbic reward circuitry, which may provide useful information for developing new strategies to treat chronic pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Chronic constrictive injury (CCI) activates contralateral ventral tegmental (VTA) dopamine (DA) neurons. (A) Timeline of CCI surgery, behavioral testing, and *in vitro* or *in vivo* recordings. (B–C) *In vitro* sample traces (B) and firing rates (C) of VTA DA neurons from sham controls and CCI mice. ** $p < 0.01$, *** $p < 0.001$; $n = 31$ –42 cells, 8 mice/group. (D) Cumulative probability distributions of *in vitro* DA neuron firing rates in ipsilateral and contralateral VTA from sham controls and CCI mice. (E) Average VTA firing rate for each mouse negatively correlated with the paw withdrawal latencies (PWLs) of contralateral hind paws. $p < 0.01$; $n = 16$ mice. (F–G) *In vivo* sample traces (F), firing rates (G, left), and bursting firing properties (G, right) of VTA DA neurons from sham controls and CCI mice. * $p < 0.05$, ** $p < 0.01$; $n = 27$ –46 cells, 10 mice/group. (H) Cumulative probability distributions of *in vivo* DA neuron firing rates in ipsilateral and contralateral VTA from sham controls and CCI mice. (I) Average VTA firing rate (left) and percentage of spikes in bursts (right) for each mouse negatively correlated with the PWLs of contralateral hind paws. $p < 0.05$; $n = 20$ mice.

Figure 2.

Inhibition of ventral tegmental (VTA) dopamine (DA) neuron firing reverses the established thermal hyperalgesia in mice with chronic constrictive injury (CCI) mice. (A) Timeline of CCI surgery, microinjection, behavioral testing, and histological staining. (B) Schematic of injector cannula tips for the experiments shown in panel C and histological plates illustrating the intra-VTA and -substantia nigra (SN) injection sites adopted from the mouse brain atlas. (C-E) Intra-VTA, but not -SN, microinjection of ZD7288 (0.1 $\mu\text{g}/0.15 \mu\text{L}$) (C), DK-AH 269 (0.6 $\mu\text{g}/0.15 \mu\text{L}$) (D), or baclofen (0.1 $\mu\text{g}/0.15 \mu\text{L}$) (E) 30 min before behavioral testing on day 7 after CCI surgery increased paw withdrawal latencies (PWLs) in CCI-affected hind paws. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$; $n = 8$ mice/group.

Figure 3.

Chronic constrictive injury (CCI) activates the contralateral ventral tegmental area (VTA)–nucleus accumbens (NAc)-projecting dopamine (DA) neurons. **(A)** Timeline of intra-NAc injection of Lumafluor, CCI surgery, c-Fos/Lumafluor double staining, and pathway-specific electrophysiological recordings. **(B)** Representative immunostaining for green fluorescent retrograde tracer and c-Fos in VTA brain sections from sham controls and CCI mice. Arrows indicate VTA neurons coexpressing the retrograde tracer and c-Fos. Scale bars, 50 μ m. **(C)** Quantitative data showing that CCI increased c-Fos expression in NAc-projecting VTA neurons. *(left)* Total numbers of VTA neurons coexpressing the retrograde tracer and c-Fos. *** $p < 0.001$; $n = 4$ mice/group. *(middle)* Percentages of c-Fos-expressing neurons in retrograde tracer-labeled VTA neurons. *** $p < 0.001$; $n = 4$ mice/group. *(right)* Percentages of fluorescent retrograde tracer-labeled neurons in c-Fos-expressing VTA neurons. * $p < 0.05$; $n = 4$ mice/group. **(D)** Schematic showing retrograde Lumafluor injection into the NAc and the labeled neurons in a VTA slice. **(E)** Sample traces *(left)* and the quantitative data *(right)* for firing rates in Lumafluor-positive DA neurons within the contralateral VTA in sham controls and CCI mice. ** $p < 0.01$; $n = 17$, 21 cells from 5 mice/group. **(F)** Sample traces *(left)* and the quantitative data *(right)* for I_h currents in Lumafluor-positive contralateral VTA–NAc DA neurons. ** $p < 0.01$, *** $p < 0.001$; $n = 10$, 12 cells from 5 mice/group. **(G)** Representative immunohistochemistry *(left)* and the quantitative data *(right)* showing that CCI increased c-Fos protein expression in the NAc, which was reversed by inhibition of VTA DA neuron firing with intra-VTA microinjection of DK-AH 269 and baclofen. DK-AH 269 (0.6 μ g/0.15 μ L) or baclofen (0.1 μ g/0.15 μ L) was injected 1.5 h before tissue sampling on day 7 after CCI surgery. *** $p < 0.0001$; 10–12 sections/mouse, $n = 6$ mice/group.

Figure 4.

In vivo optogenetic inhibition of ventral tegmental area (VTA) neurons projecting to the nucleus accumbens (NAc) reverses the established thermal hyperalgesia in affected hind paws of mice with chronic constrictive injury (CCI). (A) Schematic showing AAV2/5-CMV-H1-Cre-eYFP-SV40-WPRE (AAV2/5-Cre) injection into the NAc and injection of AAV-DIO-NpHR3.0-eYFP or its control AAV-DIO-eYFP into the VTA; VTA–NAc-projecting Cre-dependent halorhodopsin (NpHR) validation 21 days after injection. (B) Tyrosine hydroxylase (TH)/eYFP double-immunofluorescence staining validated viral expression, and quantification shows 97.8% of eYFP⁺ neurons expressed in TH (3–4 sections/mouse from three mice, bar = 100 μ m) (C) Using whole-cell current-clamp recording, *in vitro* optical stimulation (8 s on/2 s off) of an eYFP⁺ VTA–NAc neuron reliably inhibits firing activity. (D) Schematic showing retrograde AAV2/5-Cre injected into the NAc and AAV-

DIO-NpHR3.0-eYFP into the VTA and yellow light stimulation through implanted ferrule. (*E*) Timeline for intra-NAc and intra-VTA injection, CCI surgery, VTA ferrule, optical stimulation, and behavioral testing. (*F*) Acute real-time optogenetic stimulation (8 s on/2 s off) increased paw withdrawal latencies (PWLs) to thermal stimulation in the CCI-affected hind paws. ** $p < 0.01$; $n = 6, 6, 8$ mice/group.

Figure 5.

Brain-derived neurotrophic factor (BDNF) signaling in mesolimbic reward circuitry modulates nociception in mice with chronic constrictive injury (CCI). (A) Timeline of CCI surgery, ventral tegmental (VTA) *Bdnf* mRNA fluorescence *in situ* hybridization (FISH), VTA or nucleus accumbens (NAc) punches for BDNF mRNA or protein assays, NAc slices for BDNF enzyme-linked immunosorbent assay (ELISA), and VTA and/or NAc microinjections or behavioral testing. (B) FISH showed an upregulation of *Bdnf* mRNA (red) in NAc-projecting VTA neurons (green) in CCI mice. (C) Contralateral VTA and NAc BDNF mRNA expression in sham controls and CCI mice on day 7 after CCI surgery. VTA: $p < 0.05$; $n = 12$ mice/group, NAc: $n = 13$ mice/group. (D) Representative bands and quantitative data showing contralateral VTA and NAc BDNF protein expression in sham controls and CCI mice on day 7 after CCI surgery. $p < 0.05$; $n = 6$ mice/group. (E) Representative bands and quantitative data showing that the increased BDNF expression in the NAc was reversed by inhibition of VTA DA neuron firing through intra-VTA microinjections of DK-AH 269 (0.6 μg/0.15 μL) and baclofen (0.1 μg/0.15 μL). $p < 0.05$; $n = 6$ mice/group.

= 5 mice/group. **(F)** Quantitative data showing that CCI increased the BDNF released in cultured NAc slices, which was reversed by inhibition of VTA DA neuron firing through intra-VTA microinjection of DK-AH 269 (0.6 µg/0.15 µL). NAc slices were collected from a sham control group, CCI group (7 days after CCI surgery), and DK-AH 269 group (1 day after intra-VTA microinjection in CCI mice). ** $p < 0.01$, *** $p < 0.001$, $n = 4$ /group. **(G)** Intra-NAc microinjection of TrKB-Fc (20 ng/0.15 µL) but not its control IgG-Fc reversed the established hyperalgesia in CCI mice. * $p < 0.05$, ** $p < 0.01$; $n = 6-12$ mice. **(H,I)** Antinociceptive effects induced by inhibition of VTA DA neuron firing through intra-VTA microinjections of baclofen **(H)** and DK-AH 269 **(I)** were reversed by microinjection of exogenous BDNF (10 ng/0.15 µL) into the NAc. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$; $n = 6-12$ mice/group.

Figure 6.

Pathway-specific brain-derived neurotrophic factor (BDNF) signaling in ventral tegmental area (VTA)–nucleus accumbens (NAc) circuitry modulates nociception in mice with chronic constrictive injury (CCI). **(A)** Timeline of CCI surgery, and VTA and/or NAc microinjections or behavioral testing. **(B)** Inhibition of new protein synthesis by intra-VTA microinjection of anisomycin (20 μ g/0.15 μ L) induced antinociceptive effects in CCI mice. $*p < 0.05$; $n = 8$ mice/group. **(C)** Intra-NAc microinjection of BDNF (10 ng/0.15 μ L) reversed antinociceptive effects induced by intra-VTA microinjection of anisomycin (20 μ g/0.15 μ L) in CCI mice. $**p < 0.01$; $n = 8$ mice/group. **(D)** Schematic showing retrograde AAV2/5-CMV-H1-Cre-eYFP-SV40-WPRE (AAV2/5-Cre) injected into the NAc in *Bdnf^{loxP/loxP}* mice and experimental timeline for CCI surgery, validation of virus expression, reduction in BDNF protein in the VTA by genetic knockdown, and behavioral testing. **(E)** Validation of eYFP expression in VTA tyrosine hydroxylase (TH)-positive neurons 21 days after intra-NAc injections of AAV2/5-Cre in *Bdnf^{loxP/loxP}* mice and quantification showing that >96% of eYFP-expressing cells are TH⁺ (3–4 sections/mouse from three mice). **(F)** Representative bands and quantitative data showing the decrease in BDNF protein expression in the VTA 21 days after intra-NAc injection of AAV2/5-Cre in *Bdnf^{loxP/loxP}* mice. $*p < 0.05$; $n = 6$ mice/group. **(G)** Conditional knockdown of BDNF in VTA–NAc circuitry produced antinociceptive effects in CCI mice. $*p < 0.05$; $n = 6$ –10 mice.