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BDNF-mediated projection-specific regulation of depressive-like and nociceptive behaviors in mesolimbic reward circuitry

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1. Introduction

Chronic pain is undoubtedly a distressing condition and might be associated with neuropsychiatric diseases. There is increasing interest in the involvement of the mesocorticolimbic system, which is composed of dopaminergic (DA) neurons of the ventral tegmental area (VTA) and their projections to target areas, in various neuropsychiatric and neurological disorders including depression and chronic pain.¹⁻⁴ VTA DA neurons projecting to different target regions show great functional heterogeneity, which may play important roles in mediating different clinical manifestations in neurological diseases.⁵⁻⁷

It has been demonstrated that projections from the VTA to the nucleus accumbens (VTA→NAc) and from the VTA to the medial prefrontal cortex (VTA→mPFC) displayed heterogeneous intrinsic properties and played vital roles in the stress-induced depression,^{8,9} nociceptive modulation,¹⁰ and pain-related affective behaviors.¹¹ For example, a tract-tracing experiment in mice showed less than 1% overlap of VTA DA neurons that project to the mPFC and NAc. In the repeated social defeat-induced depression model, VTA→NAc DA neurons had significantly higher firing rates, yet the firing rates of VTA→mPFC DA neurons were dramatically decreased. Activation of VTA→NAc, or inhibition of the VTA

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→ mPFC, pathways induced susceptibility to social defeat stress. In contrast to these findings, in the chronic mild stress (CMS)-induced depression model, photoactivation of VTA DA neurons rescued depressive-like phenotypes and selective inhibition of VTA DA neurons induced depressive-like behaviors. Studies have also implicated the VTA→NAc pathway in nociceptive modulation. It's demonstrated that a subpopulation of VTA DA neurons exhibits excitatory responses to noxious stimuli. Furthermore, the VTA→NAc pathway has been shown to be important in pain relief, motivated behavior, and the analgesic effects of placebo and stress. Despite few experiments exploring the role of the VTA→mPFC pathway in the regulation of nociception, some studies have revealed the importance of the mPFC in nociceptive processes and pain-related emotional experiences. These findings, on the one hand, support the regulatory roles of the heterogeneous mesocorticolimbic circuit in depression and nociception, on the other hand, suggest that effects of stress and nociception on the mesocorticolimbic system are highly complex. Therefore, a better understanding of the circuit and molecular mechanisms of depression and nociception in this emotion-related system is needed.

Previous studies have indicated that brain-derived neurotrophic factor (BDNF) might be involved in the pathogenesis of depression and nociception. BDNF within the mPFC plays pivotal roles in the modulation of depression, and BDNF normalization in mPFC accounts for the effects of antidepressants. Recent studies have also explored the important modulatory roles of BDNF signaling in the mesocorticolimbic circuit in nociception and nociception-related emotions processing. These studies led to the suggestion that BDNF in the mesocorticolimbic circuit might contribute to the regulation of depression and nociception.

In this study, using a CMS-induced mouse depression model, we reveal a projection-specific modulation of depression and nociception in the mesolimbic reward circuitry and provide new insights into the neural circuits and molecular mechanisms involved in the processing of depressive and nociceptive information.

2. Materials and methods

2.1. Animals

Male Kunming mice (aged 7 weeks, Experimental Animal Center of Xuzhou Medical University, China) were housed (n=5 per cage) in a temperature-controlled vivarium, with *ad libitum* access to food and water on a 12-h light/dark cycle. All experiments were performed during the light cycle. For ethological tests, each mouse was randomly allotted for a given test alone, and all the behavioral tests were conducted by double-blind randomization in a silent room. All experimental protocols conformed to the National Institutes of Health's Guide for Care and Use of Laboratory Animals as well as the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

2.2. Chronic unpredictable mild stress procedures

The procedures were conducted to establish an animal model of depression. After 7-days of acclimation to cages, mice were subjected to 5 consecutive weeks of CMS with slight

modifications of the methods and periods of stressors. The CMS procedures consisted of water deprivation (24h), fasting (24h), day/night inversion, damp bedding (200 ml water in padding), cage tilted 45° overnight, 4°C cold bath (5 min) and tail pinching (3 min, 1.5 cm from tail tip), with 5–7 stressors involved at random each week.

2.3. Stereotaxic surgery

To prevent the impacts of stress on affective-like behaviors, the mice were anesthetized with continuous inhalation of ether (2%) throughout the intracerebral injection. Mice were mounted on a stereotaxic apparatus (Stoelting Co., Wooddale, IL, USA), followed by sterilization, scalp incision, and cranium exposure. Reagents were dissolved in normal saline and unilaterally microinjected with 33-gauge needle into (in mm): VTA (anterior-posterior, –3.5; lateral-medial, 0.5; dorsal-ventral, –4.0), mPFC (anterior-posterior, +1.8; lateral-medial, 0.4; dorsal-ventral, –2.5) or NAc shell (anterior-posterior, +1.6; lateral-medial, 0.8; dorsal-ventral, –4.5) at a rate of 0.1 µl/min followed by 5 min of rest to prevent backflow. Morphine hydrochloride (Mor, 25.5 ng/0.15 µl, H21022436, Shenyang No.1 Pharmaceutical Co. Ltd., China) or baclofen (Bac, 0.16 pg/0.15 µl, B5399, Sigma-Aldrich Co. LLC, St. Louis, MO, USA) was injected into the VTA. The retrograde green fluorescent tracer Lumafluor (0.3 µl, Lumafluor Inc., Durham, NC, USA), BDNF (10 ng/0.2 µl, B3795, Sigma-Aldrich) or TrkB-Fc (20 ng/0.2 µl, T8694, Sigma-Aldrich) was administered in the mPFC or NAc shell. For verification of injection sites, 50 µm thick brain slices with the region of the VTA, mPFC or NAc shell were cut, followed by stained with the neutral red solution (1: 2000) and inspected with light microscopy. Only successive penetrations, located in the VTA, mPFC or NAc shell were used for data analysis.

2.4. Tail suspension test (TST)

The tail suspension test was performed, with the murine caudal tip adhered on a suture and suspended 50 cm above the floor padding. Immobility was defined as cessation of any bodily movements. The immobility duration for each subject within 5 min was recorded.

2.5. Open-field test (OFT)

The OFT was performed as previously reported, except for minor modifications in the size of the arena. All mice were placed in a black plastic open-field apparatus (100 cm × 100 cm × 40 cm), illuminated by a 30W white fluorescent light 2 meters overhead. The ANY-maze tracking system (version 4.84, Stoelting Co., IL, USA) divided the field into 9 identical virtual squares, and the number of line crossings within 10 min was recorded.

2.6. Social interaction test

The social interaction test was performed as previously reported, with minor modifications of the position of the social target. Each mouse was placed in a cubic box (40 cm × 40 cm × 40 cm). A virtual arena (12 cm × 10 cm), defined as ‘interaction zone’ was set on the box floor adjacent to another box (10 cm × 10 cm × 20 cm) housing an unacquainted target mouse, with a wire-mesh window in between. The total duration of each experimental mouse in the interaction zone within 3 min was calculated with TopScan Lite software (Clever Sys., Inc., Reston, VA, USA).

2.7. Sucrose preference test

The sucrose preference test was performed for a 24 h duration as from mouse placement (5:00 p.m.) in individual cages, with *ad libitum* access to 1% sucrose solution or tap water in two bottles (50 ml) fitted with ball-point sipper tubes. To obviate location preference, the bottles were exchanged every 12 h, with the liquids weighed at the end of the accommodation (5:00 pm the next day) and sucrose preference calculated as a percentage of sucrose solution intake relative to total consumption. In this study, 4h, 1d and 3d timepoints of the sucrose preference indicated the calculated sucrose preference from 4 hours, 1 day and 3 days after intracranial injection to 24 hours later.

2.8. Paw withdrawal latency test

The paw withdrawal latency (PWL) test was conducted as previously described. Each mouse was displaced in a transparent plastic box (7 cm × 9 cm × 11 cm) located on 3 mm-thick plexiglass pane of IITC Plantar Analgesia Meter (IITC Life Science Inc., CA, USA) for acclimation of 1 h. The calcaneal pad of hind paw was heated by a portable focused heater from underneath. The PWL was defined as the period as from the commencement of focused heating to the onset of paw licking or lifting, with the thermal intensity adjusted to achieve basal PWL in 8 to 14 s and an automatic cutoff preset to 25 s to avoid localized insults. The tests were in triplicate for each mouse at an interval of 5 min, with the mean PWL calculated as the thermal nociceptive threshold.

2.9. Ex vivo cell-attached electrophysiology

Cell-attached recordings were obtained from VTA DA neurons in acute brain slices. 5 days after microinjection of retrograde green fluorescent microbeads (Lumafluors, Lumafluor, Inc) in either mPFC or NAc shell, mouse was anesthetized (10% chloral hydrate, 300 mg/kg, i.p.) and then decapitated, following which 300 µm VTA slices were sectioned with a vibrating microslicer (VT1000S, Leica, Germany) in cold sucrose artificial cerebrospinal fluid (aCSF), comprising (in mM): 254 sucrose, 1.25 NaH₂PO₄, 10 D-glucose, 24 NaHCO₃, 2 CaCl₂, 2 MgCl₂ and 3 KCl (oxygenated with 95% O₂ and 5% CO₂). Following incubation at 35°C in aCSF for 1h, sections were transferred into a recording chamber with aCSF (at 32–33°C, flow velocity = 2.5 ml/min). Herein, aCSF differed from sucrose aCSF in 128 mM NaCl in the former and sucrose in the latter. Patch pipettes (3–5 MΩ) for cell-attached recordings were filled with an internal solution comprising (in mM): 115 K-gluconate, 10 phosphocreatine, 10 HEPES, 1.5 MgCl₂, 20 KCl, 2 Mg-ATP and 0.5 GTP. VTA-mPFC or VTA-NAc putative DA neurons were identified by the fluorescence of Lumafluor and then characterized by their electrophysiological traits: regular triphasic action potential waveforms, prolonged action potential duration, and low firing frequencies. Signals were band-pass filtered at 300 Hz–1 kHz and Bessel filtered at 10 kHz by a Multiclamp 700B amplifier.

2.10. Immunohistochemistry

Mice subjected to microinjection of retrograde green fluorescent tracer Lumafluor in either mPFC or NAc shell were anesthetized (10% chloral hydrate, 300 mg/kg, i.p.), followed by intracardial perfusion with 4% paraformaldehyde in PBS (pH 7.4). The brains were post-

fixed overnight, transferred to 30% sucrose for 48 hours, and sectioned (30 μ m) using a freezing microtome (Leica Microsystems, model VT1000S). The slices were blocked in 10% donkey serum and exposed overnight to mouse anti-tyrosine hydroxylase (TH) (1:500, MAB318, Millipore, CA, USA.). The antibodies were labeled with donkey anti-mouse Alexa 594 (1:400, R37115, Invitrogen). Sections were imaged at $\times 20$ magnification for analyses. Cell counting was performed manually.

2.11. In vivo electrophysiology

Spontaneous firing from putative VTA DA neurons in mice was recorded. Under chloral hydrate (10%, 300 mg/kg, i.p.) anesthesia, a glass-coated tungsten microelectrode with an impedance of 0.6–1.0 M Ω (Frederick Haer) was advanced toward the VTA through a guiding tube by a micromanipulator. The coordinates of VTA were as follows (in mm): anterior-posterior, -3.30 – 3.70 ; lateral-medial, 0.40 – 0.60 ; and dorsal-ventral, -3.5 – 4.5 . The multi-channel workstation (Alpha Lab SnR, Alpha Omega) was employed for the collection, amplification, and filtration of the action potentials of neurons. Identification of spontaneous firings of VTA DA neurons was based on the electrophysiological properties: (I) prolonged duration (>1.2 ms); (II) triphasic action potentials with negative waveforms; (III) low firing frequencies (<10 Hz); (IV) spontaneous single and burst (an interval < 80 ms between spikes) firings. Steady *in vivo* firings were recorded prior and subsequent to morphine injection (25.5 ng/0.15 μ l) in the VTA for 5 min, respectively.

2.12. Western blotting analyses

Tissue blocks of mPFC and NAc shell were punched 4 h and 1 h after intra-VTA injection respectively. The tissues were homogenized in lysis buffer containing protease and phosphatase inhibitors, followed by centrifugation (13000 rpm, 15 min, 4°C). With the supernatants collected and protein concentrations measured, the supernatants were adjusted to the identical protein concentration across groups. The samples (40 μ g protein per lane) were electrophoresed by 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). The membranes were thrice rinsed and blocked in 5% w/v skim milk for 2h at 25°C, followed by incubation in primary polyclonal rabbit anti-BDNF (1:1000, sc-546, Santa Cruz, USA,) or polyclonal rabbit anti- β -Tubulin (1:1000, E021040-01, Earthox, USA) antibody overnight at 4°C. Afterwards, the bands were rinsed and incubated in alkaline phosphatase-labeled secondary antibody (1:1000, AP-1000, VECTOR, USA) (2h, 25°C), and were visualized by a BCIP/NBT kit (S3771, Promega, USA). We detected a band of approximately 15 kDa, indicating truncated BDNF. β -Tubulin served as the standard of comparison and the gray scale intensity of bands was analyzed by ImageJ software.

2.13. BDNF quantification by ELISA

Quantification of BDNF release in mPFC and NAc shell was carried out 4h and 1h after microinjection of morphine into the VTA, respectively. Following the procedures of preparing brain slices for *ex vivo* cell-attached electrophysiology, coronal slices of 300 μ m were obtained while submerged in a slush of sucrose aCSF. The mPFC and NAc shell slices were trimmed from the whole coronal slices and were then transferred and maintained in aCSF saturated with carbogen at 37°C for 45 minutes. 50 μ l of the cultured supernatant of mPFC and NAc shell slices for every sample was collected for assay.

BDNF protein levels in the cultured supernatant of mPFC and NAc shell slices were analyzed using the Mouse Brain Derived Neurotrophic Factor ELISA KIT (MBS724249, MyBioSource) according to the manufacturer's protocol. Briefly, 50 μ l of standards or samples were added and incubated for 1 h at 37°C in 96-well Microtiter Plates coated with anti-BDNF monoclonal antibody. To quantitatively determine the amount of BDNF in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for BDNF was added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm.

2.14. Data analysis and statistics

Statistical analyses were conducted with GraphPad Prism 5.0. Values in graphs are expressed as mean $\pm$ SEM. Student's *t*-test was used to compare the electrophysiological data. One-way ANOVA was conducted to analyze the western blotting and *ex vivo* recording data, followed by the Tukey's *post-hoc* tests. Two-way repeated measures ANOVA was performed to analyze the two factors (grouping and timing) of behavioral and electrophysiological data, followed by Bonferroni *post-hoc* tests when appropriate. Sample sizes are indicated in the figure legends, and differences of $p < 0.05$ were considered statistically significant.

3. Results

3.1. The changes of firing rates in the VTA→mPFC and VTA→NAc DA neurons in CMS mice

CMS mice showed significant depressive-like phenotypes, i.e. decreased line crossings during the OFT (Fig. 1A1), prolonged immobility duration during the TST (Fig. 1A2), decreased social interaction (Fig. 1A3) and diminished sucrose preference (Fig. 1A4). However, following CMS, no significant differences in thermal nociceptive threshold (paw withdraw latency, PWL) was found (Fig. 1B). It is well accepted that VTA DA neuronal firing rates show great heterogeneity, which underlies its extensive effects on regulating different behavioral responses. Therefore, it was critical for us to elucidate the changes of firing rates in VTA DA neurons projecting to mPFC or NAc shell following CMS. To answer this question, the retrograde green fluorescent tracer was infused into mPFC or NAc shell to label the mPFC- or NAc shell-projecting VTA neurons (Supplementary Fig. 1, 2), respectively, and the firing rates in the lumafluor-labeled neurons were recorded (Fig. 1C). Immunohistochemical analysis showed that most of the lumafluor-labeled neurons were TH-positive neurons (Fig. 1D, E). Projection-specific electrophysiological data showed that the firing rates of VTA→mPFC putative DA neurons were significantly inhibited in CMS mice when compared with stress-naïve (Ctrl) mice (Fig. 1F), yet no significant differences were observed in the VTA→NAc DA neurons between the both groups (Fig. 1G). These data indicated that CMS resulted in circuit-specific modulation of firing activity in the mesocorticolimbic circuits.

3.2. Elevated responses to morphine in the VTA DA neurons in CMS mice

Our ex vivo recording data is also supported by a previous report that in vivo firing rates of VTA DA neurons were significantly reduced in CMS mice. Therefore, enhancement of VTA DA neuronal firing should relieve CMS-induced depressive-like behaviors. A previous study has demonstrated that acute microinjection of morphine (60 ng/ 0.06 μ l) can activate VTA DA neurons in stress-naïve rats. Thus, we wanted to first explore the effects of intra-VTA administration of morphine on the in vivo firing rates of putative VTA DA neurons in anesthetized CMS mice (Supplementary Fig. 3). Here, intra-VTA microinjection of morphine chloride (25.5 ng/0.15 μ l) was used as a tool to activate putative VTA DA neurons. We found that CMS stress causes a reduction in the firing rates of VTA DA neurons (Fig. 2A, B). Furthermore, intra-VTA infusion of morphine hydrochloride (25.5 ng/0.15 μ l) markedly elevated the firing rates of VTA DA neurons in CMS mice, but not in stress-naïve mice (Fig. 2A, B).

To determine if there is a circuit-specific effect of morphine-induced activation of VTA DA neurons in CMS mice, we performed projection-specific ex vivo recordings with bath application of morphine (0.22 μ g/ μ l). We first replicated our results that CMS mice showed the decreased firing rates in the VTA→mPFC DA neurons when compared with Ctrl mice (Fig. 2C, D), and there are no differences in firing rates of the VTA→NAc DA neurons between CMS and Ctrl mice (Fig. 2E, F). We next revealed that morphine infusion significantly increased the firing rates of both the VTA→mPFC and VTA→NAc DA neurons in CMS mice, but not stress-naïve mice (Fig. 2C–F). These data suggest that persistent stress induced latent sensitization of VTA DA neurons, which could be unmasked by exogenous morphine administration.

3.3. Activation of VTA DA neurons relieved depressive-like behaviors but produced thermal nociceptive responses in CMS mice

Next, we assessed the effects of activation of VTA DA neurons on depressive-like behaviors and nociception in CMS mice undergoing intra-VTA injection of morphine (25.5 ng/0.15 μ l). Present results found that VTA administration of morphine relieved depressive-like behaviors at 4 h and 1 d after administration, as evidenced by increased line crossings in OFT (Fig. 3A1), decreased immobility duration in TST (Fig. 3A2), prolonged interaction in the social interaction test (Fig. 3A3) and enhanced sucrose preference (Fig. 3A4). CMS-induced depressive-like behaviors appeared again at 3 d following activation of VTA DA neurons by morphine (Fig. 3A1–4). Surprisingly, intra-VTA injection of morphine produced a transient thermal nociceptive response, as indicated by the decreased PWL in CMS mice at 0.5 and 1h after intra-VTA infusion (Fig. 3B–D). To further confirm this apparent paradoxical finding, we pharmacologically increased VTA DA neuronal firing rates through another mechanism by VTA microinjection of a low concentration of baclofen (0.16 pg/0.15 μ l), which has been demonstrated to increase excitability of VTA DA neurons, and then performed depressive-like behaviors and thermal nociceptive tests. A similar finding was also found after baclofen injection (Fig. 3E–H). These data suggest that pharmacological activation of VTA DA neurons in CMS mice relieves depressive-like behaviors, yet induces nociceptive responses.

3.4. BDNF in the VTA→mPFC pathway mediated regulation of depressive-like behaviors in CMS mice

BDNF in the mesolimbic reward system has been shown to be involved in the repeated social defeat-induced depressive-like behaviors.²⁷ Here, we explored whether BDNF signaling in this emotional circuitry is implicated in the regulation of depressive-like behaviors and nociceptive responses by activation of VTA DA neurons underlying CMS-induced state of depression.

Western blot analysis showed that BDNF expression in mPFC was downregulated in CMS mice, and was reversed 4 h after intra-VTA injection of morphine (Fig. 4A1). Moreover, using ELISA assay, we found that the basal release of BDNF in cultured slices of the mPFC was significantly decreased in CMS mice as compared with control mice, which was increased 4 h after intra-VTA injection of morphine (Fig. 4A2). With these results demonstrating that morphine administration in the VTA leads to increased release of BDNF in the mPFC in CMS mice, we wanted to determine whether exogenous administration of BDNF (10 ng/0.2 μ l) in the mPFC could change depressive-like phenotypes and nociception in CMS mice.

Our data showed that injection of exogenous BDNF in mPFC induced antidepressant-like effects in CMS mice including increased line crossings in the OFT (Fig. 4B1), attenuated time spent immobile in the TST (Fig. 4B2), reversed social avoidance during a social interaction test (Fig. 4B3) and increased sucrose preference (Fig. 4B4). However, BDNF administration into mPFC did not alter thermal nociceptive threshold in CMS mice (Fig. 4C, D). These data indicate that modulation of BDNF signaling in mPFC might participate in the regulation of depressive-like behaviors, but not nociception, induced by CMS.

To further illustrate the relationship between the upregulation of BDNF expression in the mPFC and the relief of depressive-like behaviors by activation of VTA DA neurons, we blocked BDNF signaling by injection of TrkB-Fc (20 ng/0.2 μ l) into the mPFC 10 min prior to morphine or saline infusion into the VTA. Assessment of depressive-like behaviors revealed that there were no significant differences in line crossings in OFT (Fig. 4E1), immobility duration in TST (Fig. 4E2), social interactions (Fig. 4E3) and sucrose consumption (Fig. 4E4) between CMS mice receiving both injections of TrkB-Fc in mPFC and saline or morphine in the VTA. These data suggest that inhibition of BDNF signaling in the mPFC could abolish the antidepressant-like effects observed with morphine activation of VTA DA neurons. However, blocking of BDNF signaling in mPFC failed to change the thermal nociceptive responses by VTA DA neuronal activation in CMS mice, as indicated by shortened PWL (Fig. 4F–H). Together, these findings revealed that BDNF signaling in the VTA→mPFC pathway regulates depressive-like behaviors, but not nociceptive responses, following activation of VTA DA neurons in CMS state.

3.5. BDNF in VTA→NAc pathway mediated regulation of nociceptive behaviors in CMS mice

Next, we investigated whether BDNF signaling in the VTA→NAc pathway is implicated in the regulation of depressive-like behaviors and nociceptive responses by activation of VTA

DA neurons underlying state of depression. Measurement of BDNF expression in the NAc shell showed no significant alterations following CMS. However, intra-VTA injection of morphine significantly upregulated BDNF levels in NAc shell in CMS mice, but not in control mice (Fig. 5A1). Quantification of BDNF by ELISA found that intra-VTA injection of morphine also increased the release of BDNF in NAc shell in CMS, but not in control, mice (Fig. 5A2).

To further study the role of BDNF signaling in the VTA→NAc pathway in the regulation of depressive-like behaviors and nociceptive responses, we next tested the effects of exogenous administration of BDNF (10 ng/0.2 μ l) in NAc shell on depressive-like behaviors and thermal nociceptive threshold in CMS mice. The results indicated that no significant alterations were found in depressive-like behavioral tests including the line crossings during OFT (Fig. 5B1), immobility duration in TST (Fig. 5B2), social interaction behaviors (Fig. 5B3) and sucrose preference (Fig. 5B4) following BDNF injection in NAc shell. However, intra-NAc shell injection of BDNF decreased PWL in CMS, but not in control mice (Fig. 5C, D).

To explore the relationship between the upregulation of BDNF expression in the NAc shell and VTA DA activation-induced thermal nociceptive responses in CMS mice, we blocked BDNF signaling by injection of TrkB-Fc (20 ng/0.2 μ l) into the NAc 10 min prior to morphine or saline infusion into the VTA in CMS mice. The results found that TrkB-Fc injection in NAc shell did not change the antidepressant-like effects by morphine-induced activation of VTA DA neurons (Fig. 5E1–4). However, the thermal nociceptive responses induced by intra-VTA injection of morphine were prevented by intra-NAc shell injection of TrkB-Fc (Fig. 5FH). Collectively, these findings indicated that BDNF signaling in the VTA→NAc pathway might act as a potential modulator for nociception, but not for depressive-like behaviors, following activation of VTA DA neurons in CMS state.

4. Discussion

Our behavioral, electrophysiological and neuropharmacological results consistently demonstrated that CMS decreased VTA DA neuronal firing activity in the VTA→mPFC, but not in the VTA→NAc, pathway, and the VTA DA neurons in CMS mice showed an enhanced response to morphine administration when compared with stress-naïve mice. These data suggested that CMS induced the heterogeneous regulation of VTA DA neuronal activity in a circuit-dependent manner. Our study is consistent with previous findings that VTA DA neuronal activity was inhibited by aversive stimuli and optical activation of VTA DA neurons rescued CMS-induced depression phenotype. Additionally, a significant reduction in burst activity of VTA DA neurons was detected in the Flinders Sensitive Line (FSL) rat, a genetic animal model of depression. However, contradictory results were found in chronic restraint and social defeat stress-induced depression models. These studies reflect the heterogeneous properties of VTA DA neurons and their responses to different forms of stressors. Further study of VTA DA neuronal activity in an anatomical-, input- or output-dependent manner might contribute to interpreting these paradoxical findings.

Next, we found that relief of depressive-like behaviors by intra-VTA injection of morphine in CMS mice was intriguingly accompanied by a significant thermal nociceptive response. The similar effects were also revealed after intra-VTA injection of low-dose of baclofen, which preferentially inhibited GABAergic neurons resulting in the disinhibition of VTA DA neurons and increase of its firing activity. Previous studies have shown that NAc shell, but not mPFC, projecting VTA DA neurons displayed large hyperpolarization-activated current (I_h). Compared with VTA→mPFC DA neurons, VTA→NAc DA neurons displayed lower membrane excitability and slower pacemaker activity. These intrinsic modulatory heterogeneities in VTA DA neurons projecting to different target areas may account for the varying physiological or pathological characteristics. Mediation of depression by VTA→mPFC pathway and of nociception-related responses by VTA→NAc pathway in our study is also consistent with previous researches. For example, optogenetic inhibition of the VTA→mPFC pathway induced social avoidance behavior in C57/BL6 mice subjected to subthreshold social defeat. Some studies have demonstrated that the NAc was required for mediating nociceptive modulation by VTA DA neurons, and activation of the VTA to NAc dopamine signaling contributed to both pain-related positively and negatively reinforced behaviors.

Based on the previous studies, we focused on BDNF in the VTA→mPFC and VTA→NAc pathways to explore the molecular mechanisms underlying the antidepressant-like and nociceptive effects by activation of VTA DA neurons in CMS mice. We demonstrated that normalized BDNF functions of the VTA→mPFC pathway are essential to the relief of depressive-like behaviors. Conversely, sequestration of endogenous BDNF with TrkB-Fc in the mPFC could reverse the antidepressant-like effects by activation of the VTA DA neurons in CMS mice. Previous studies have shown that repeated stress induces dendritic atrophy in the rat mPFC. Significant downregulation of BDNF expression in the mPFC was confirmed by autopsy in suicide victims with depression. Additionally, antidepressants may exert their efficacy by upregulating BDNF expression in rodents and the observed normalized BDNF levels in humans. BDNF/TrkB and its downstream signaling molecules might account for the modulatory effects of BDNF in depressive phenotypes. For example, BDNF regulated GSK-3β through PI3K/AKT signaling. Chronic stress decreased the p-Ser9-GSK-3β expression in mPFC, which strengthened GSK3β functions and consequently declined gene expression and synaptic functions. Vice versa, exogenous BDNF might rescue the stress-induced changes in the BDNF-GSK-3β pathway and facilitate synaptic functions. Also, the BDNF/ TrkB -MAPK/ERK cascade has been explored to be involved in the modulation of neuroplasticity and stress responses. Intra-mPFC injection of BDNF might activate this pathway and stimulate downstream mammalian target of rapamycin (mTOR) signaling, which increased synaptic proteins expression and synaptic transmission. Collectively, CMS-induced depressive-like behavior is associated with the down-regulation of BDNF in the mPFC, while the activation of VTA DA neurons might facilitate the release of BDNF to the mPFC so as to attenuate depression.

We also found that activation of VTA DA neurons in CMS mice promoted the release of BDNF protein in the NAc shell. This result was consistent with enhancement of NAc BDNF expression induced by optogenetic activation of VTA→NAc neurons in mice subjected to subthreshold social defeat stress. In addition to this, infusion of exogenous BDNF into the

NAc shell induced a significant thermal nociceptive response, and blockage of BDNF signaling in the NAc shell antagonized the intra-VTA morphine-induced nociception, suggesting a vital role of BDNF in VTA→NAc pathway in nociceptive modulation. The NAc regulates the perception of noxious information and descending pain modulatory circuits.²⁹ Imaginal studies revealed activated NAc in humans with chronic pain.³⁰ Imaging studies also demonstrated activated NAc in rats with peripheral nerve injury. NAc serves to predict the value of a noxious thermal stimulus at its onset and offset. Stimulation of the NAc provided relief from central poststroke pain in humans. Our recent research also found that chronic constriction injury (CCI) of sciatic nerve upregulated the BDNF expression in the contralateral NAc. Moreover, inhibition of BDNF synthesis in the VTA or selective knockdown of BDNF in the VTA→NAc pathway induced antinociceptive effects in CCI mice. Together with these findings, our study provides evidence that VTA→NAc pathway BDNF signaling is a critical modulator of nociception in CMS mice. Despite the reported mechanisms that BDNF within the VTA→NAc circuit might mediate depressive-like behaviors in social defeat mice, it is still less clear about the functions of BDNF in chronic mild stress, especially at the neural circuit levels. Social defeat stress alters the motivation for social interaction in rodents, which induces depressive-like phenotypes,³¹ and might also relate to pertinent aspects of anxiety disorders. Given to the differences of schema and intensity of stressors involved in CMS model and social defeat paradigm, it is relatively difficult to compare the roles of BDNF in the two animal models.

To help us to draw the conclusion from the present data, three points should be further clarified. First, CMS did not affect nociceptive threshold. The results were consistent with recent reports that basal thermal and mechanical pain threshold were not changed in CMS mice.³² However, studies also showed increased thermal and mechanical nociceptive thresholds in rats that underwent chronic stress, as well as reduced nociceptive responses in CMS rats. Additionally, CMS might induce thermal hyperalgesia and mechanical allodynia. These observations indicate that the effects of stress on pain threshold might depend on the procedures for CMS, and further studies are needed to explore the underlying mechanisms.

Second, both in vivo VTA recording and ex vivo VTA→mPFC DA neurons recording showed decreased firing rates in CMS mice. In vivo recording data might be collected from mixed populations of VTA DA neurons projecting to multiple target regions (e.g., the mPFC, the NAc, the hippocampus, the amygdala). Although chronic stress did not change the activity of VTA→NAc DA neurons, CMS decreased the firing rates of VTA→mPFC DA neurons. Also, we cannot exclude the possibility that chronic stress might decrease the activity of the hippocampus- or the amygdala- projecting VTA DA neurons. Therefore, during in vivo recording, the comprehensive outcomes of CMS could be decreased firing rates of VTA DA neurons. Present study is also comparable to our previous findings that social defeat significantly increased the firing rates of VTA→NAc DA neurons, yet dramatically decreased VTA→mPFC DA neurons, in brain slices. However, the overall in vivo VTA firing rates were notably increased in social defeat mice. Together, CMS decreased the firing rates of VTA→mPFC DA neurons ex vivo and the activity of VTA DA neurons in vivo.

The third is the finding of intra-VTA delivery of morphine-induced hyperalgesia, rather than analgesia. Although there was the evidence showing that intra-VTA injection of morphine (3.0 µg/0.5 µl/side) inhibited formalin pain scores in rats, few studies explored the effect of intra-VTA morphine on the basal nociceptive threshold. Our results showed that intra-VTA morphine (25.5 ng/0.15 µl) did not change the basal thermal nociceptive threshold in control mice, but induced a significant thermal nociceptive response in CMS mice. Three possible reasons might account for the discrepancy: (1) A much lower dose of morphine was used in the present study. The previous finding has shown that low in comparison with usual doses of morphine maybe have different effects and mechanisms in modulating nociception. (2) The dose of morphine used in the present study increased firing activity in only stressed VTA DA neurons, suggesting a context gating mechanism. (3) The difference in animal species and the methods for assessment of nociceptive behaviors.

In conclusion, we report that activation of VTA DA neurons under CMS state produced the antidepressant-like effects via BDNF signaling in the VTA→mPFC pathway, accompanied by thermal nociceptive responses mediated by BDNF signaling in the VTA→NAc pathway (Fig. 6). Our findings revealed a novel role for projection-specific regulation of depressive-like and nociceptive behaviors by BDNF signaling in mesolimbic reward circuitry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary

BDNF signaling in the VTA→mPFC and VTA→NAc circuits regulates depressive phenotypes and nociceptive behavior in mice exposed to chronic mild stress.

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Figure 1.

Chronic unpredictable mild stress induced depressive-like phenotypes and decreased firing rates of the VTA-mPFC, but not VTA-NAc DA neurons. (A1–4) Marked depressive-like behaviors in CMS-treated mice (Two-way repeated measures ANOVA displayed stress $\times$ time epoch interaction in OFT: $F_{(1, 10)} = 151.13$; in TST: $F_{(1, 10)} = 20.8$; in social interactive test: $F_{(1, 10)} = 9.19$; in sucrose preference test: $F_{(1, 11)} = 7.30$; Bonferroni *post-hoc* test showed changed behaviors in CMS group relative to the Ctrl group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 5–7$ mice/group). (B) No significant difference was observed in PWL between CMS and control mice (stress $\times$ time interaction: $F_{(1, 10)} = 0.02$, $p = 0.8944$; Bonferroni *post-hoc* test: $p > 0.05$ vs. Ctrl group, $n = 6$ mice/group). (C) Schematic showing retrograde green fluorescent microbeads (Lumafluor) injected into mPFC or NAc shell and retrograded toward the soma of projecting-specific VTA putative DA neurons (left). A trace displayed the ex vivo action potential of the Lumafluor-labeled putative DA neuron in VTA (right). (D) Confocal images showing expression of green Lumafluor, which was intra-mPFC injected, in VTA DA neurons. Quantification of green Lumafluor expressions in the VTA neurons showed that $81 \pm 3\%$ of Lumafluor-expressing neurons were TH+ ($n = 10$ sections from 5 mice). Scale bar: 20 μm . (E) Images showing the distribution of green Lumafluor-labeled neurons and TH+ neurons in the VTA from mice injected with retrograde green fluorescent Lumafluor in the NAc shell. Quantification of green Lumafluor expressions showed that $87 \pm 2\%$ of Lumafluor-expressing neurons were TH+ ($n = 10$ sections from 5 mice). Scale bar: 20 μm . (F) The activity of VTA-mPFC putative DA neurons was inhibited by stressors ($t_{18} = 5.091$, *** $p < 0.001$ vs. Ctrl mice, $n = 10$ cells from 8 mice/group). (G) No significant alterations of the firing frequency were observed

from VTA-NAc putative DA neurons in CMS-mice ($t_{18} = 1.089$, $p > 0.05$ vs. Ctrl mice, $n = 10$ cells from 7 mice/group).

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Figure 2.

Elevated responses to morphine in VTA-mPFC and VTA-NAc DA neurons of CMS mice. (A) Sample traces showing in vivo spontaneous firings of VTA DA neurons. (B) In vivo electrophysiological measures revealed reduced firing frequency of putative VTA DA neurons in CMS mice, which were elevated by morphine hydrochloride injected into the VTA (stress $\times$ drug treatment interaction: $F_{(1, 17)} = 9.88$, $p < 0.01$; Bonferroni *post-hoc* test showed decreased spontaneous firing frequency in CMS mice relative to the Ctrl group mice before intra-VTA morphine injection, $**p < 0.01$, $***p < 0.001$, $n = 7, 12$ cells from 7, 11 mice). (C) Sample traces showing spontaneous firing events of VTA-mPFC putative DA neurons from VTA slices. (D) The ex vivo spontaneous firing frequency of VTA-mPFC putative DA neurons was reduced in CMS mice, and then moderately normalized by morphine, in only CMS mice ($F = 22.1$, $p < 0.001$; Tukey's *post-hoc* test: $***p < 0.001$ vs. CMS-Sal mice, $n = 17, 17, 16, 16$ cells from 12 mice each). (E) Sample traces recorded from VTA-NAc shell putative DA neurons from VTA slices. (F) CMS did not alter the firing frequency of the VTA-NAc putative DA neurons, yet only VTA-NAc shell putative DA neurons of CMS mice were over-activated by morphine ($F = 25.7$, $p < 0.001$; Tukey's *post-hoc* test: $***p < 0.001$ vs. CMS-morphine mice, $n = 14, 14, 17, 17$ cells from 8–9 mice each).

Figure 3.

Relief of depressive-like behaviors and induction of hyperalgesia by intra-VTA infusion of morphine or baclofen in CMS mice. (A1) In the OFT, morphine increased line crossings in CMS mice at 4h and 1d (stress $\times$ time epoch interaction: $F_{(12, 112)} = 2.99$; Bonferroni *post-hoc* test displays increased line crossings in CMS-Mor(VTA) group relative to the CMS-Sal(VTA) group, $*p < 0.05$, $n = 8$ mice/group). (A2) In the TST, morphine decreased immobility duration in stressed mice at 4h and 1d (stress $\times$ time epoch interaction: $F_{(12, 112)} = 6.5$; Bonferroni *post-hoc* test shows decreased immobility duration in CMS-Mor(VTA)

group relative to the CMS-Sal(VTA) group, $**p < 0.01$, $***p < 0.001$; $n = 8$ mice/group). (A3) In the social interaction test, morphine prolonged the interaction of CMS mice at 4h (stress $\times$ time epoch interaction: $F_{(12, 112)} = 2.58$; Bonferroni *post-hoc* test demonstrates increased interactive behaviors in CMS-Mor(VTA) group relative to the CMS-Sal(VTA) group, $***p < 0.001$, $n = 8$ mice/group). (A4) In the sucrose preference test, morphine increased the 1% sucrose preference in CMS mice at 4h and 1d (stress $\times$ time epoch interaction: $F_{(12, 112)} = 3.25$; Bonferroni *post-hoc* test displays increased sucrose consumption in CMS-Mor(VTA) group relative to the CMS-Sal(VTA) group, $*p < 0.05$, $**p < 0.01$, $n = 8$ mice/group). (B) Intra-VTA infusion of morphine reduced the PWL in CMS mice at 0.5 and 1h (stress $\times$ time epoch interaction: $F_{(12, 112)} = 4.18$; Bonferroni *post-hoc* test indicates hyperalgesia in CMS-Mor(VTA) group relative to the CMS-Sal(VTA) group, $***p < 0.001$, $n = 8$ mice/group). (C) The experimental groups for studying the effects of intra-VTA injection of morphine on depressive-like behaviors and nociceptive phenotype. (D) Experimental timeline. (E1) In the OFT, baclofen increased line crossings in CMS mice at 4h (stress $\times$ time epoch interaction: $F_{(12, 112)} = 10.12$; Bonferroni *post-hoc* test reveals more locomotion of CMS-Bac(VTA) group than the CMS-Sal(VTA) group, $*p < 0.05$, $n = 8$ mice/group). (E2) In the TST, baclofen decreased the immobility duration in CMS mice at 4h (stress $\times$ time interaction: $F_{(12, 112)} = 5.99$; Bonferroni *post-hoc* test: $**p < 0.01$ vs. CMS-Sal(VTA) group, $n = 8$ mice/group). (E3) In the social interaction test, baclofen increased the interaction of CMS mice at 4h (stress $\times$ time interaction: $F_{(12, 112)} = 3.9$; Bonferroni *post-hoc* test: $*p < 0.05$ vs. CMS-Sal(VTA) group, $n = 8$ mice/group). (E4) In the sucrose preference test, baclofen increased the 1% sucrose preference in CMS mice at 4h (stress $\times$ time interaction: $F_{(12, 112)} = 8.28$; Bonferroni *post-hoc* test: $**p < 0.01$ vs. CMS-Sal(VTA) group, $n = 8$ mice/group). (F) Intra-VTA infusion of baclofen reduced the PWL in stressed mice at 0.5 and 1h (stress $\times$ time interaction: $F_{(12, 112)} = 2.38$; Bonferroni *post-hoc* test: $**p < 0.01$ vs. CMS-Sal(VTA) group, $n = 8$ mice/group). (G) The experimental groups for exploring the effects of intra-VTA injection of baclofen on depressive-like behaviors and nociception. (H) Experimental timeline, including CMS paradigm, stereotaxic surgery and behavioral tests.

Figure 4.

Intra-mPFC injection of exogenous BDNF attenuated depressive-like behaviors, and blocking BDNF signaling in mPFC prevented the relief of depressive-like phenotypes by VTA DA neurons activation in CMS mice. (A1) BDNF expression levels in mPFC were down-regulated in CMS mice, which were reversed by intra-VTA injection of morphine ($F = 6.185$, $p < 0.05$; Tukey's *post-hoc* test: * $p < 0.05$ vs. CMS-Sal mice, $n = 3$ mice/group). (A2) BDNF release in mPFC was decreased in CMS mice, and was rescued by microinjection of morphine in VTA (One-way ANOVA test, $F = 113.7$, $p < 0.001$; Tukey's *post-hoc* test: * $p < 0.05$ vs. CMS-Sal mice, $n = 3$ mice/group).

0.05, *** $p < 0.001$ vs. CMS-Sal mice, $n = 3$ mice/group). (B1) In the OFT, BDNF injection increased the line crossings in CMS mice at 4h (stress $\times$ time epoch interaction: $F_{(12, 112)} = 1.89$, $p = 0.0431$; Bonferroni *post-hoc* test: ** $p < 0.01$ vs. CMS-Sal(mPFC) group mice, $n = 8$ mice/group). (B2) In the TST, two-way repeated measures ANOVA demonstrated the stress-by-time epoch interaction (interaction of the experimental-group factor and time point factor, $F_{(12, 112)} = 12.51$, $p < 0.01$; Bonferroni *post-hoc* test revealed reduced escape-related behavior in the CMS-BDNF(mPFC) group relative to the CMS-Sal(mPFC) group: ** $p < 0.01$, *** $p < 0.001$, $n = 8$ mice/group). (B3) BDNF infusion reversed the social avoidance performance at 4h (stress $\times$ time epoch interaction: $F_{(12, 104)} = 2.13$, $p = 0.021$; Bonferroni *post-hoc* test showed more social interactive behavior in the CMS-BDNF(mPFC) group than the CMS-Sal(mPFC) group: ** $p < 0.01$, $n = 7-8$ mice/group). (B4) BDNF administration increased the sucrose preference in CMS mice at 4h and 1d (stress $\times$ time epoch interaction: $F_{(12, 104)} = 6.66$, $p < 0.001$; Bonferroni *post-hoc* test: ** $p < 0.01$, *** $p < 0.001$ vs. CMS-Sal(mPFC) mice, $n = 7-8$ mice/group). (C) Intra-mPFC injection of exogenous BDNF failed to alter the thermal PWL in both normal and depressive-like mice (stress $\times$ time epoch interaction: $F_{(12, 112)} = 1.27$, $p > 0.05$, $n = 8$ mice/group). (D) The experimental groups for testing the effects of intra-mPFC injection of exogenous BDNF on depressive-like behaviors and nociception. (E1) In the OFT, TrkB-Fc reduced the line crossings which would otherwise have been increased by morphine administration in VTA in CMS mice (Two-way repeated measures ANOVA demonstrated notable depressive-like behavior in CMS mice, main effect of stress: $F_{(3, 112)} = 33.75$, $p < 0.001$; Bonferroni *post-hoc* test showed insignificant difference between the CMS-Sal(VTA)-TrkB-Fc(mPFC) group and the CMS-Mor(VTA)-TrkB-Fc(mPFC) group: $p > 0.05$, $n = 8$ mice/group). (E2) In the TST, the depressive-like mice with TrkB-Fc injection in mPFC displayed prolonged immobility duration (Two-way repeated measures ANOVA revealed main effect of stress: $F_{(3, 112)} = 23.48$, $p < 0.001$; Bonferroni *post-hoc* test: $p > 0.05$ vs. CMS-Sal(VTA)-TrkB-Fc(mPFC) group, $n = 8$ mice/group). (E3) TrkB-Fc administration deprived the CMS mice of morphine-promoted social interactions (Two-way repeated measures ANOVA indicated depressive-like status of CMS mice, with main effect of stress: $F_{(3, 112)} = 21.94$, $p < 0.001$; Bonferroni *post-hoc* test: $p > 0.05$ vs. CMS-Sal(VTA)-TrkB-Fc(mPFC) group, $n = 8$ mice/group). (E4) Sucrose preference was reduced in CMS-treated mice in the sucrose preference test (Two-way repeated measures ANOVA showed main effect of stress: $F_{(3, 112)} = 34.31$, $p < 0.001$; *post-hoc* test: $p > 0.05$ vs. CMS-Sal(VTA)-TrkB-Fc(mPFC) group, $n = 8$ mice/group). (F) In the PWL test, intra-VTA injection of morphine shortened PWL at 0.5h, 1h and 2h in CMS mice, with the TrkB-Fc actions making it void (stress $\times$ time epoch interaction: $F_{(12, 112)} = 6.65$, $p < 0.001$; main effect of stress: $F_{(3, 112)} = 16.19$, Bonferroni *post-hoc* test: * $p < 0.05$, *** $p < 0.001$ vs. CMS-Sal(VTA)-TrkB-Fc(mPFC) group, $n = 8$ mice/group). (G) The experimental groups for intra-VTA infusion of morphine posterior to TrkB-Fc injected into mPFC. (H) Experimental timeline.

Figure 5.

Intra-Nac shell injection of exogenous BDNF induced thermal hyperalgesia without affecting depression, and blockage of BDNF signaling in Nac shell prevented the thermal hyperalgesia induced with morphine activation of VTA DA neurons in CMS mice. (A1) CMS induced no significant alterations of BDNF expression in Nac shell, which was further upregulated by morphine injected in VTA ($F = 12.89$, $p < 0.001$; Tukey's *post-hoc* test: ** $p < 0.01$ vs. CMS-Sal mice, $n = 3$ mice/group). (A2) One-way ANOVA indicated significant enhancement of BDNF release in Nac shell by intra-VTA injection of morphine in CMS

mice. ($F = 13.89$, $p < 0.01$; Tukey's *post-hoc* test: $*p < 0.05$, $**p < 0.01$ vs. CMS-Mor mice, $n = 3$ mice/group). (B1) In the OFT, BDNF injection failed to augment the number of line crossings (Two-way repeated measures ANOVA indicated depressive-like phenotypes in CMS mice, with main effect of stress: $F_{(3, 135)} = 31.2$, $p < 0.001$; Bonferroni *post-hoc* test failed to reveal increased locomotion of the CMS-BDNF(NAc shell) group relative to the CMS-Sal(NAc shell) group: $p > 0.05$, $n = 7-8$ mice/group). (B2) In the TST, BDNF did not abbreviate immobility duration (main effect of stress: $F_{(3, 140)} = 49.06$, $p < 0.001$; Bonferroni *post-hoc* test: $p > 0.05$ vs. CMS-Sal(NAc shell) group, $n = 8$ mice/group). (B3) Injection of BDNF in NAc shell failed to affect the social behaviors in depressive-like mice (Two-way repeated measures ANOVA showed main effect of stress: $F_{(3, 108)} = 19.61$, $p < 0.001$; Bonferroni *post-hoc* test: $p > 0.05$ vs. CMS-Sal(NAc shell) group, $n = 7-8$ mice/group). (B4) Exogenous BDNF showed no effects on the sucrose consumption in CMS mice (main effect of stress: $F_{(3, 112)} = 18.89$, $p < 0.001$; Bonferroni *post-hoc* test: $p > 0.05$ vs. CMS-Sal(NAc shell) group, $n = 8$ mice/group). (C) Intra-NAc shell injection of BDNF shortened PWL in CMS mice at 0.5h and 1h (stress $\times$ time epoch interaction: $F_{(12, 112)} = 5.3$, $p < 0.001$; main effect of stress: $F_{(3, 112)} = 8.2$, Bonferroni *post-hoc* test indicated hyperalgesia in CMS-BDNF(NAc shell) group relative to the CMS-Sal(NAc shell) group: $***p < 0.001$, $n = 8$ mice/group). (D) The experimental groups for intra-NAc shell injection of exogenous BDNF. (E1) In the OFT, with TrkB-Fc infused into NAc shell prior to morphine injection into VTA, the number of line crossings was augmented at 4h (Two-way repeated measures ANOVA displayed main effect of stress: $F_{(3, 112)} = 15.88$, $p < 0.001$; Bonferroni *post-hoc* test showed increased locomotion of the CMS-Mor(VTA)-TrkB-Fc(NAc shell) group relative to the CMS-Sal(VTA)-TrkB-Fc(NAc shell) group: $*p < 0.05$, $n = 8$ mice/group). (E2) In the TST, the immobility duration in CMS micewas decreased at both 4h and 1d (stress $\times$ time interaction: $F_{(12, 112)} = 8.03$, $p < 0.001$; main effect of stress: $F_{(3, 112)} = 29.08$, $p < 0.001$; Bonferroni *post-hoc* test: $**p < 0.01$, $***p < 0.001$ vs. CMS-Sal(VTA)-TrkB-Fc(NAc shell) group mice, $n = 8$ mice/group). (E3) The depressive-like mice exhibited extended social interactions at 4h and 1d (Two-way repeated measures ANOVA demonstrated stress $\times$ time interaction: $F_{(12, 112)} = 7.94$, $p < 0.001$; main effect of stress: $F_{(3, 112)} = 33.46$, $p < 0.001$; Bonferroni *post-hoc* test: $**p < 0.01$, $***p < 0.001$ vs. CMS-Sal(VTA)-TrkB-Fc(NAc shell) group mice, $n = 8$ mice/group). (E4) Sucrose preference was restored in depressive-like mice at 4h and 1d (stress $\times$ time interaction: $F_{(12, 112)} = 5.74$, $p < 0.001$; main effect of stress: $F_{(3, 112)} = 19.89$, $p < 0.001$; Bonferroni *post-hoc* test: $*p < 0.05$, $**p < 0.01$ vs. CMS-Sal(VTA)-TrkB-Fc(NAc shell) group mice, $n = 8$ mice/group). (F) In the PWL test, sequential administrations of TrkB-Fc in NAc shell and morphine in VTA failed to induce hyperalgesia in CMS mice (Two-way repeated measures ANOVA indicated insignificant stress-by-time epoch interaction: $F_{(12, 112)} = 1.43$, $p = 0.1643$; Bonferroni *post-hoc* test: $p > 0.05$ vs. CMS-Sal(VTA)-TrkB-Fc(NAc shell) group mice, $n = 8$ mice/group). (G) The experimental groups for testing roles of intra-NAc shell infusion of TrkB-Fc in the antidepressant-like effects and hyperalgesia by morphine injection in VTA. (H) Detailed schematic of experiment, consisting of CMS paradigm, stereotaxic surgery and ethology.

Figure 6.

Projection-specific modulation of depression and nociception in mesolimbic reward circuitry in CMS-induced depressive states. Chronic unpredictable stress decreases the excitability of VTA-mPFC DA neurons, diminishes BDNF signaling in mPFC and induces depressive-like phenotypes. Activation of VTA-mPFC DA neurons reversed depressive-like behaviors through normalization of BDNF signaling in mPFC. Activation of VTA-NAc DA neurons induced nociceptive behavior through the increase of BDNF release in NAc shell.