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Overexpression of BDNF in the ventral tegmental area enhances binge cocaine self-administration in rats exposed to repeated social defeat

Junshi Wang^{#1,2,+}, Ryan M. Bastle^{#2}, Caroline E. Bass³, Ronald P. Hammer Jr.^{1,2}, Janet L. Neisewander², and Ella M. Nikulina¹

¹University of Arizona College of Medicine, Phoenix, AZ

²School of Life Sciences, Arizona State University, Tempe, AZ

³School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY

These authors contributed equally to this work.

Abstract

Stress is a major risk factor for substance abuse. Intermittent social defeat stress increases drug self-administration (SA) and elevates brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area (VTA) in rats. Intra-VTA BDNF overexpression enhances social defeat stress-induced cross-sensitization to psychostimulants and induces nucleus accumbens (NAc)

FosB expression. Therefore, increased VTA BDNF may mimic or augment the development of drug abuse-related behavior following social stress. To test this hypothesis, adeno-associated virus (AAV) was infused into the VTA to overexpress either GFP alone (control) or GFP+BDNF. Rats were then either handled or exposed to intermittent social defeat stress before beginning cocaine SA training. The SA acquisition and maintenance phases were followed by testing on a progressive ratio (PR) schedule of cocaine reinforcement, and then during a 12-h access “binge” cocaine SA session. BDNF and FosB were quantified postmortem in regions of the mesocorticolimbic circuitry using immunohistochemistry. Social defeat stress increased cocaine intake on a PR schedule, regardless of virus treatment. While stress alone increased intake during the 12-h binge session, socially-defeated rats that received VTA BDNF overexpression exhibited even greater cocaine intake compared to the GFP-stressed group. However, VTA BDNF overexpression alone did not alter binge intake. BDNF expression in the VTA was also positively correlated with total cocaine intake during binge session. VTA BDNF overexpression increased

FosB expression in the NAc, but not in the dorsal striatum. Here we demonstrate that VTA BDNF overexpression increases long-access cocaine intake, but only under stressful conditions.

Corresponding author: Ella M. Nikulina, PhD, Department of Basic Medical Sciences, University of Arizona College of Medicine – Phoenix, 425 N 5th St. Phoenix, AZ 85004 USA, Telephone: 602-827-2168, Fax: 602-827-2130, nikulina@email.arizona.edu.

⁺Current address: Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

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Statement of Interest

None.

Therefore, enhanced VTA-BDNF expression may be a facilitator for stress-induced increases in drug abuse-related behavior specifically under conditions that capture compulsive-like drug intake.

Keywords

brain-derived neurotrophic factor; social defeat stress; cocaine self-administration; nucleus accumbens; deltaFosB

1. Introduction

The propensity to develop compulsive drug taking varies across individuals (Warner, 1995), and the transition from recreational to compulsive drug use is greatly influenced by the interplay between intrinsic genetic factors and environmental stimuli, such as stress (Bardo et al., 2013). Stress is an etiological factor in substance use disorders that contributes to individual differences in the vulnerability to develop drug dependence (Sinha, 2001). Among many stress models in laboratory animals, the social defeat model has strong ethological value because it is a natural, non-adaptable psychosocial stressor that has a high degree of face validity for modeling the aversive consequences of peer competition in humans. For instance, human drug addicts experiencing this type of psychosocial stress exhibit stronger drug craving and have higher rates of dependence and relapse (Anderson et al., 2006; Reed et al., 2006). Similarly, rats exposed to intermittent social defeat exhibit enhanced motivation to self-administer cocaine and binge-like behavior, as well as behavioral sensitization to psychostimulants (Nikulina et al., 2004; Quadros and Miczek, 2009).

Both drugs of abuse and stress activate mesolimbic circuitry (Wise, 1998), and the ventral tegmental area (VTA) is a critical anatomical substrate linking stress-induced dysregulation with vulnerability to psychostimulants (Nikulina et al., 2014; Nikulina et al., 2012; Wang et al., 2013). Intermittent social defeat stress induces long-lasting expression of brain-derived neurotrophic factor (BDNF) in the VTA (Fanous et al., 2010). In the mesolimbic circuitry, BDNF is synthesized by dopaminergic neurons in the VTA, and is transported anterogradely to the nucleus accumbens (NAc) (Altar et al., 1997). BDNF is essential for many forms of long-term synaptic plasticity (Thoenen, 1995). Increased BDNF in the VTA leads to enhanced sensitivity of dopaminergic neurons in the VTA to excitatory input (Pu et al., 2006). Also, increased VTA BDNF is associated with enhanced drug-seeking behavior that occurs during withdrawal from cocaine self-administration (Grimm et al., 2003; Lu et al., 2004). Furthermore, we previously found that BDNF overexpression in the VTA prolongs social defeat stress-induced cross-sensitization to amphetamine and enhances sensitization induced by repeated amphetamine injections (Wang et al., 2013). Taken together, these findings suggest that increased VTA BDNF might be a potential risk factor causing increases in drug abuse-related behaviors, and may further enhance stress-induced vulnerability to develop compulsive drug taking behaviors. Importantly, the BDNF Val66Met polymorphism is associated with abnormal intracellular trafficking and activity-induced secretion of BDNF (Egan et al., 2003), and increases the propensity to develop anxiety and depressive disorders under stress in humans (Chen et al., 2006). Thus, intrinsic enhancement of VTA BDNF

levels may act a risk factor to develop characteristics of drug dependence, especially in stressed individuals.

FosB is a member of the Fos family of transcription factors whose induction in the NAc occurs in response to chronic exposure to various drugs of abuse or stress (Hope et al., 1994; Perrotti et al., 2004). Accumulation of FosB in the NAc is thought to underlie the long-term nature of addiction and has been directly linked to addiction-related behaviors (Kelz et al., 1999; Nestler, 2008). Furthermore, we previously observed that VTA BDNF overexpression alone increases FosB levels in the NAc (Wang et al., 2013). Considering that FosB expression in the NAc leads to increased sensitivity to drug abuse (Kelz et al., 1999), it is possible that VTA BDNF levels may contribute to the increased propensity of stressed individuals to develop drug dependence.

In order to test whether VTA BDNF increases drug abuse-related behaviors, we used viral-mediated gene transfer to overexpress VTA BDNF in both handled and stressed rats. This approach allowed us to test whether VTA BDNF overexpression alone can recapitulate the effects of stress and/or exacerbate the effects of stress on cocaine self-administration during 1) acquisition, 2) maintenance, 3) tests on a progressive ratio schedule, and 4) a test on a 12-h access “binge” session. In addition, we measured FosB expression in the NAc and dorsal striatum, respectively, following behavioral testing.

2. Material and Methods

A timeline of all procedures is depicted in Figure 1A.

2.1. Animals

Male Sprague Dawley experimental rats (total n = 68; Charles River Laboratories, Hollister, CA) weighing 250-300 g were housed individually in a humidity- and temperature-controlled room on a 10:14-h reversed light/dark cycle (lights on at 2100 h) with food and water available *ad libitum*. Stimulus aggressive “resident” Long-Evans rats (retired breeders, Charles River Laboratories, Hollister, CA) weighing ~500 g upon arrival, were housed with a female Sprague Dawley rat for at least two weeks before use. Experimental rats were acclimated to laboratory conditions and handling for 7 days. Animal care and housing conditions were consistent with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). Surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee at Arizona State University. All efforts were made to minimize suffering and limit the number of animals used in the experiments.

Viral vector—Pseudotyped AAV2/10 vectors were used in all experiments, as described previously (Wang et al., 2013). Briefly, the AAV2/10 rep/cap plasmid provided the AAV2 replicase and AAV10 capsid genes (Gao et al., 2002), while adenoviral helper functions were supplied by the pHelper plasmid (Stratagene, La Jolla, CA). The AAV plasmids contain a transgene cassette, which consists of the cytomegalovirus (CMV) promoter and either the gene for rat BDNF fused to enhanced green fluorescent protein (GFP) or GFP alone, followed by a combined intron/polyadenylation signal derived from SV40. These elements are flanked by two AAV2 inverted terminal repeats. A standard triple transfection

protocol was used to generate the helper-free pseudotyped AAV2/10 vectors as follows (Xiao et al., 1998): the three plasmids were co-transfected into HEK-293 cells (Stratagene, La Jolla, CA) via calcium phosphate precipitation; cells were harvested 48 h later, resuspended in DMEM, freeze-thawed with dry ice-ethanol slurry three times and centrifuged to produce a clarified cell lysate. The resulting viral stocks were stored at -80°C and are referred to as GFP (control virus) and BDNF (BDNF+GFP overexpressing virus). The effect of these AAV vectors has previously been validated in both neurochemical and behavioral assays (Wang et al., 2013).

2.2. Surgery

Rats received an analgesic (buprenorphine, 0.05 mg/kg, SC) and antibiotic (Cefazolin, 30 mg/kg, SC) prior to induction of isoflurane anesthesia (2-3%; Abbott Laboratories, North Chicago, IL), vaporized in oxygen and delivered through a plastic nose cone. Catheters were then implanted into the jugular vein as described previously (Pockros et al., 2011).

Rats were then positioned in a stereotaxic frame (David Kopf Instruments; Tujunga, CA) where two holes were drilled at stereotaxic coordinates (AP -5.1 mm; ML ± 2.15 mm from bregma) on the surface of skull. Hamilton syringes (Model 7105 KH; Reno, NV) with 24-gauge injector tips were lowered through the holes bilaterally at a 10° angle until the needle tips reached the VTA (AP -5.1 mm; ML ± 0.6 mm; DV -8.8 mm from bregma (Paxinos and Watson, 2007), and 0.5 μl of virus was infused bilaterally into the VTA for 10 min at a speed of 0.05 $\mu\text{l}/\text{min}$. Following viral infusion, the syringes remained at the infusion sites for 5 min allow for diffusion into tissue.

The cannula end of the catheter was then anchored to the skull using dental acrylic cement and four small anchor screws. The head and neck incisions were sutured and treated with a topical antibiotic, and the rats received an anti-inflammatory medication (meloxicam; 1.0 mg/kg, SC). A flexible obturator made from Tygon tubing was fitted over the cannula to protect the catheter. Patency of the catheters was maintained throughout the experiment by daily flushing with 0.1 ml timentin (66.67 mg/ml; bioWORLD, Dublin, OH) in sterile saline solution containing 70 units/ml heparin sodium. Catheter patency was tested periodically with 0.8 g methohexital sodium (Brevital; Sigma-Aldrich, St. Louis, MO), a dose that produces rapid loss of muscle tone only when administered IV. Following surgery, rats were left to recover for 7 days in their home cages and were handled and weighed daily.

2.3. Social stress procedure

One week following surgery, rats were exposed to social defeat stress or handling, as previously described (Nikulina et al., 2012). Briefly, after removing the female from the resident's cage, the experimental intruder rat was placed into the resident's home cage under a stainless steel mesh protective cage (15 \times 25 \times 15 cm) for 5 min to expose to threat from a resident. The protective cage was removed, and the resident displayed aggressive behavior. "Defeat" ensued when the intruder exhibited a supine posture for at least 4 s, which occurred within 2–5 min. Following "defeat," the intruder was placed under the protective cage for an additional 15 min for further stress without physical contact and was then returned to the home cage. Experimental rats assigned to social defeat received a total of 4 exposures across

10 days. Control rats were handled and weighed on the days their counterparts were defeated and weighed.

2.4. Self-administration apparatus

Self-administration training and testing were conducted in Plexiglas operant conditioning chambers (20 × 28 × 20 cm; Med Associates, St. Albans, VT) individually encased within a ventilated, sound-attenuating cabinet. A stimulus light was mounted above the designated active lever. An infusion pump containing a 10-ml syringe was located outside of the cabinet. Tygon tubing connected to the syringe to a liquid swivel (Instech, Plymouth Meeting, PA) suspended above the operant conditioning chamber and connected the outlet of the swivel to the catheter via Tygon tubing. The latter tubing ran through a metal spring leash (Plastics One, Roanoke, VA) fastened onto the plastic screw of the cannula that was anchored to the animal's head.

2.5. Acquisition and maintenance

After recovery from surgery and one week after the last episode of social stress, self-administration (SA) training commenced during daily sessions. The following SA procedures and cocaine doses were chosen based on previous work that showed an increase in both progressive ratio and binge intake following repeated social defeat stress (Covington and Miczek, 2005; Miczek and Mutschler, 1996). Within 5 min after a rat was placed into an SA chamber, the beginning of the session was signaled by insertion of the levers. Initially rats were given the opportunity to self-administer cocaine (0.75 mg/kg/0.1 ml, IV) on a fixed ratio (FR) 1 schedule of reinforcement for at least 2 sessions. During these sessions, pressing the active lever was reinforced by the light cue, followed 1 s later by a 6-s cocaine infusion, and then a 23-s timeout period during which responses did not count toward the next ratio requirement. Responses on the inactive lever were recorded, but did not result in any consequences. Each daily session terminated after the delivery of 15 infusions or 5 h of access, whichever came first. We defined acquisition as the total session time to reach criterion (i.e., time that elapsed from the start of the first session until the rats met the criterion of obtaining 15 infusions during a given session). After the rat self-administered 15 cocaine infusions/session for 2 consecutive days, the response requirement was gradually increased from FR1 to FR5 over the next 3-5 days. The rats were then maintained on a FR5 schedule (15 infusions per day or 5 h access) for 5 consecutive sessions.

2.6. Progressive ratio

Following acquisition and maintenance (FR5) for 5 consecutive daily sessions, rats underwent self-administration according to a progressive ratio (PR) schedule of cocaine reinforcement (0.375 mg/kg/infusion) every other day until completing 3 PR sessions, with maintenance sessions (0.75 mg/kg/infusion, FR5, 15 infusions) on alternating days. The ratio to attain each successive cocaine infusion was calculated according to the formula $5 * e^{(0.2n)} - 5$, where n represents the number of infusions received during the session (Richardson and Roberts, 1996). The progressive response requirement was as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, etc. The last completed ratio before failing to attain a reinforcer within 1 h was defined as the break point. The number of

active lever presses and intake was averaged over the three PR sessions for each rat and used in the analysis.

2.7. 12-h Binge session

After the final PR session, each rat was allowed one maintenance session of cocaine self-administration (0.75 mg/kg/infusion, FR5, 15 infusions). The next day, a prolonged access protocol was implemented starting at approximately 0800 h (i.e., 1 h after the start of the dark cycle). Each rat was allowed continuous access to cocaine (0.375 mg/kg/infusion, FR5) during the entire 12-h session.

2.8. Perfusion and tissue processing

Ten days after the 12-h binge session, all rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, IP) and perfused transcardially with 10 ml of 10% heparin in 0.1 M phosphate-buffered saline (pH 7.4) followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were post-fixed for 1 h in the same fixative at 4°C, followed by graded sucrose concentrations in 0.1 M PBS at 4°C for 24 h before sectioning. Brains were sectioned at 20 µm in a cryostat at -22°C, and thaw-mounted onto glass slides (Superfrost Plus; Fisher Scientific; Waltham, MA). Sections were collected from +1.8 to +0.8 mm from bregma for the NAc, and -4.8 to -5.6 mm from bregma for the VTA (Paxinos and Watson, 2007).

2.9. Immunohistochemistry

Sections were washed in 0.05 M potassium phosphate-buffered saline (KPBS), then blocked for 1 h in 10% normal donkey serum and 0.4% Triton X-100 in 0.05 M KPBS, and incubated with primary antibody: BDNF (AB1779SP, 1:3,000 dilution; Millipore; Temecula, CA) or FosB (SC-48, 1:5,000 dilution; Santa Cruz Biotechnology, Inc.; Santa Cruz, CA). The FosB antibody used here targets the N terminal of the FosB protein contained in both FosB and FosB isoforms. However in this experiment, FosB-like labeling would primarily capture accumulation of FosB; FosB expression is transient and only FosB persists for days after stimulation (Perrotti et al., 2004). Following incubation with primary antibody for 48 h at 4°C, slides were washed in 0.05 M KPBS and incubated for 1 h in biotin-conjugated goat anti-rabbit serum (1:200 dilution in blocking solution, Vectastain ABC kit; Vector Laboratories; Burlingame, CA). After washing in 0.05 M KPBS, sections were incubated with avidin-biotin-peroxidase complex (Vectastain ABC kit) for 1 h, then washed again and developed using DAB chromogen with nickel intensification (DAB Peroxidase Substrate kit, Vector Laboratories). After dehydration in graded concentrations of ethanol and xylene, coverslips were applied.

2.10. Image analysis

Tissue sections were examined for the presence of GFP (Fig. 1B) and chromogen reaction product; selected areas were captured and digitized using a color digital video camera interfaced to the microscope. Viral infusion-induced GFP signal (Ex 488 nm, Em 509 nm) was detected unequivocally using a GFP filter set in a fluorescence microscope without immunolabeling. Immediately after sections of VTA were collected onto slides, the location

of GFP signal in each rat was observed directly under the fluorescence microscope to localize the infusion site. Rats with an incorrect viral infusion location were excluded from the study. For immunohistochemical quantification, a cell profile inside the target brain region ($900 \times 1200 \mu\text{m}$) was considered labeled if its pixel intensity was more than 2 standard deviations greater than that of background within the target area, as calculated by Stereo Investigator software (MBF Biosciences; Williston, VT). At least three adjacent sections were selected in which the number of labeled cells was quantified and averaged for each brain region in each animal.

2.11. Statistical Analysis

Cocaine self-administration behavior and immunohistochemical data were analyzed by two-way ANOVAs [between subjects factors: virus (AAV-BDNF vs. AAV-GFP) and stress exposure (handled vs. stressed)]. Comparisons of interest were performed to test whether stress or VTA BDNF overexpression alone would enhance measures compared to GFP handled controls, as well as whether VTA BDNF overexpression and stress together would enhance measures compared to stress alone. The alpha levels for these comparisons were adjusted using the Bonferroni correction for multiple comparisons (alpha level/number of comparison). All data are reported as mean \pm SEM. Results were considered significant at $p < 0.05$.

During acquisition, the session was prematurely terminated in a subset of animals ($n=20$) due to an electrical surge that occurred during the first day of acquisition (FR1). Acquisition data from those rats were therefore excluded. Their cocaine self-administration behavior during maintenance (FR2 to FR5), however, was indistinguishable from other rats and was included, thus leading to a variability of sample size between acquisition and all other analyses. Two rats were excluded due to failure to complete the cocaine self-administration phase and four rats were excluded from the study due to misplaced viral infusions. For the immunohistochemical data, local tissue damage led to different sample sizes per group in adjacent brain regions (NAc core and shell, dorsal striatum); final n 's are reported in the figure captions.

3. Results

3.1. Localization of viral infusion sites in the VTA

Successful viral infusion sites were localized by the presence of GFP labeling primarily in the rostral VTA (-4.8 to -5.6 mm from bregma; Fig. **1B**).

3.2. VTA BDNF nor social defeat stress affect acquisition or maintenance of cocaine self-administration

To measure acquisition, we calculated the number of hours each rat took before successfully reaching the criterion for schedule advancement on the FR1 schedule (i.e., 15 infusions/session). We found no significant interaction or main effects between the four groups (Table 1). In addition, during maintenance of cocaine self-administration (i.e., 5 days of FR5 sessions), there was no significant difference across the four groups (Table 1).

3.3. Social defeat stress, but not VTA BDNF overexpression, enhances cocaine self-administration

During PR testing, significant main effects of stress on active lever presses ($F_{(1, 58)} = 6.276$, $p < 0.05$) and infusions received ($F_{(1, 58)} = 9.471$, $p < 0.01$) were present where the stressed groups exhibited higher rates of responding and intake than handled control groups (Fig. 2). In contrast to the significant main effect of stress, neither VTA-BDNF overexpression alone nor combined with stress had any significant effect on rates of responding or intake compared to their respective GFP controls (Fig. 2A, B). No significant main effects of virus or interactions between the two factors were observed with these measures, nor were there differences in inactive lever pressing across groups (Table 1).

3.4. VTA BDNF overexpression with social defeat stress enhances intake during a 12-h binge session

For total active lever pressing and infusions received, we found significant main effects of stress ($F_{(1, 58)} = 15.591$ and 13.128 , respectively, $ps < 0.01$) and virus ($F_{(1, 58)} = 10.042$ and 6.733 , respectively, $ps < 0.05$), but no interaction was observed between the two factors. In agreement with previous findings of a stress-induced increase in binge cocaine self-administration (Covington and Miczek, 2001; Miczek et al., 2011), the GFP-stressed group had significantly greater active lever pressing (Bonferroni-corrected t-test, $p < 0.0167$) and a non-significant trend toward an increase in infusions (Bonferroni-corrected t-test, $p = 0.055$) compared to the GFP-handled group (Fig. 3A, 3B). The BDNF-handled group did not exhibit an increase compared to the GFP-handled group ($ps > 0.05$). Furthermore, the BDNF-stressed group had the highest rate of responding and cocaine intake across the session (Fig. 3C, 3D) and had significantly greater rates of responding and intake compared to the GFP-stressed group (Bonferroni-corrected t-tests, $ps < 0.0167$). No significant main effects or interaction were found with inactive lever pressing ($ps > 0.05$).

3.5. VTA BDNF levels correlate with binge intake

Exposure to social stress and/or intra-VTA infusion of AAV-BDNF induced significantly greater BDNF expression in the VTA after cocaine self-administration (Figs. 4A, 4B), indicated by significant main effects of virus ($F_{(1, 48)} = 38.454$, $p < 0.001$) and stress ($F_{(1, 48)} = 15.046$, $p < 0.001$), but no interaction between the two factors. Consistent with our previous study (Fanous et al., 2010), social defeat stress alone increased VTA BDNF expression compared to GFP-handled controls (Fig. 4B, Bonferroni-corrected t-test, $p < 0.0167$). Similarly, the BDNF-handled group had greater VTA BDNF expression than the GFP-handled group (Fig. 4B, Bonferroni-corrected t-test $p < 0.0167$), demonstrating overexpression that is in agreement with our previous finding that this viral construct is able to increase VTA BDNF levels compared to controls for at least 45 days (Wang et al., 2013). Furthermore, the BDNF-stressed group had significantly more BDNF labeling compared to the GFP-stressed group (Fig. 4B; Bonferroni-corrected t-test $p < 0.0167$). Moreover, there was a significant positive correlation between VTA BDNF labeling and lever pressing and intake during the binge session (Fig. 4D, 4E; Pearson correlations: $r_{(52)} = 0.507$, $p < 0.001$; $r_{(52)} = 0.499$, $p < 0.001$, respectively).

3.6. VTA BDNF increased FosB expression in the NAc, but not in the dorsal striatum

Groups with VTA BDNF overexpression exhibited significantly greater FosB labeling in both the NAc core (main effect of virus, $F_{(1, 39)} = 6.967, p < 0.05$) and shell (main effect of virus, $F_{(1, 43)} = 4.515, p < 0.05$) compared to groups that received AAV-GFP (Fig. 5). No main effect of stress or interaction between the two factors was found in either region. In contrast, VTA BDNF overexpression had no effect on FosB expression in the dorsal striatum (Fig. 6). Interestingly, stressed groups exhibited lower FosB expression in the dorsal striatum than handled groups (main effect of stress $F_{(1, 41)} = 8.076, p < 0.01$). No significant interaction was observed between the two factors.

4. Discussion

The present study examined whether VTA BDNF overexpression could recapitulate or exacerbate stress-induced increases in cocaine self-administration. In agreement with previous findings (Quadros and Miczek, 2009), we found that social defeat stress increased motivation to self-administer cocaine under a PR schedule of reinforcement. Despite the findings that VTA BDNF overexpression alone did not alter responding or intake during PR testing, it greatly potentiated cocaine self-administration during the 12-h binge session in rats with previous stress exposure. Given that elevated VTA BDNF expression is only one of many consequences of stress-induced neuroplasticity, it appears to be insufficient to mimic the effect of stress on cocaine abuse-related behaviors. Instead, VTA BDNF overexpression may facilitate the development of stress-induced neuroplasticity involved in increased binge cocaine self-administration. VTA BDNF overexpression also increased FosB expression in the NAc after chronic cocaine self-administration, providing additional insight into the changes to the mesocorticolimbic system that occurred following our manipulations.

4.1. VTA BDNF overexpression specifically enhanced cocaine intake in stressed rats in 12-hour binge, but not during PR

In humans, binge patterns of cocaine intake are characterized by closely spaced administrations over long periods of time when a large supply of drug is available and can lead to fatal overdose (Pottier et al., 1992). The 12-h binge session in the present study was designed to capture this pattern of cocaine use in humans. In accordance with previous work (Covington and Miczek, 2001; Miczek et al., 2011), we found that social defeat stress alone increased binge responding. However, the less pronounced increase we observed may be due to our shorter binge access period (i.e. 12 h vs. 24 h) or to strain differences across studies. Furthermore, while VTA BDNF overexpression alone had no impact on binge measures, rats that received both VTA BDNF overexpression and social defeat stress exhibited the greatest amount of responding and intake during the binge session, even above stress alone. These findings suggest that increased VTA BDNF expression augments stress-induced neuroplasticity that contributes to increases in binge-like cocaine intake.

In addition to increasing local BDNF expression, stress induces multiple neurochemical changes in the VTA, including increasing dopamine output (Boyson et al., 2014; Miczek et al., 2011), enhancing glutamatergic receptor expression (Covington et al., 2008; Wang et al., 2014), and inducing CRF release in the VTA (Wang et al., 2005). The respective

intracellular signaling of stress and BDNF in the VTA involves phosphorylation of ERK, a major downstream target of the BDNF signaling cascade, as VTA ERK inhibition prior to social defeat prevents the development of stress-induced cross-sensitization and escalation of cocaine SA during binge (Yap et al., 2015). Stress also plays a gating role for increased BDNF signaling in mesolimbic pathways (Walsh et al., 2014). Since BDNF is a well-known facilitator of synaptic plasticity (Figurov et al., 1996), elevated BDNF in the VTA could potentiate some specific facets of stress-induced neuroplasticity. Indeed, increased VTA-originated BDNF signaling is necessary for the susceptibility to the aversive effect of social defeat stress (Berton et al., 2006; Krishnan et al., 2007). Specifically, the afferent BDNF signaling in the NAc appears to be critical for stress-induced effects on drug abuse-related behavior. For instance, we previously found that knockdown of the BDNF receptor, tropomyosin-related kinase B (TrkB), in the NAc prevented stress-induced locomotor cross-sensitization to amphetamine and the associated neurochemical changes, such as increases in BDNF and GluA1 in the VTA, and FosB in the NAc shell (Wang et al., 2014). Importantly, it was shown that BDNF-TrkB signaling in the NAc is critical for drug self-administration (Graham et al., 2007; Graham et al., 2009). These results suggest that BDNF signaling in the VTA and the NAc is critical for stress-induced neuroplasticity in the mesolimbic circuitry and behavioral changes. Therefore, this may explain the pattern of cocaine intake during the 12-h binge session where VTA BDNF overexpression only increased intake under stress conditions, but had no effect on stress-naïve rats.

Although some studies have reported social stress-induced enhancement of cocaine self-administration acquisition (Haney et al., 1995), our lack of acquisition effects is consistent with several others in the field that used similar procedures (Covington and Miczek, 2001; Quadros and Miczek, 2009). Surprisingly, we did not find an augmented increase of drug intake during PR testing as we had observed during the 12-h binge session in the BDNF-stressed group. This discrepancy suggests that PR and binge exposure tap into different aspects of drug abuse with distinct mechanisms. While PR effects demonstrate the motivational and reinforcing efficacies attributed to drugs, binge effects may reflect the compulsiveness to self-administer drugs under low effort, extended access conditions. The former involves assessment of action-outcome, whereas the latter reflects habitual behavior (Everitt, 2014). Furthermore, social defeat stress has inconsistent effects on PR, where some reports have illustrated an enhancement (Covington and Miczek, 2005; Quadros and Miczek, 2009), while others have found no effect (Miczek et al., 2011). Therefore, social defeat stress-induced neuroplasticity may have a stronger impact on compulsive binge-taking behavior and increased VTA BDNF may enhance this neuroplasticity and exacerbate the phenotype.

4.2. Neurochemical changes in rats treated with stress and/or VTA BDNF overexpression after cocaine self-administration

Our previous work has shown that intermittent social defeat stress induces long-lasting increases in BDNF expression in the VTA (Fanous et al., 2010; Wang et al., 2013). Similarly, here we found significantly higher VTA BDNF expression in rats exposed to social defeat stress than handled rats even following a history of cocaine self-administration and abstinence, which alone is known to increase VTA BDNF expression (Schmidt et al.,

2012). Our data suggests that social defeat stress-induced increases in VTA BDNF were not masked by cocaine self-administration and 10 days of forced abstinence. Importantly, we found that VTA BDNF levels significantly correlated with cocaine intake during the binge session. A positive correlation between BDNF gene polymorphism and drug dependence (Uhl et al., 2001) or depression induced by life stress has been reported in human studies (Hosang et al., 2014). Taken together, these data suggest that augmented VTA BDNF may contribute to enhanced vulnerability to develop a compulsive-like pattern of drug intake following psychosocial stress.

Along with increased BDNF expression in the VTA, we found that VTA BDNF overexpression enhanced FosB expression in the NAc core and shell, which is consistent with our previous work (Wang et al., 2013). In response to chronic stress or drugs of abuse,

FosB gradually accumulates in the NAc and persists longer than other of Fos-family proteins (Nestler, 2008; Perrotti et al., 2004). In contrast, FosB expression in the NAc shell and core diminishes during long-term withdrawal from chronic cocaine self-administration (Larson et al., 2010). Therefore, the relative increase of NAc FosB expression we observed following 10 days of abstinence is likely due to persistently enhanced BDNF signaling originating from the VTA. For instance, VTA-originated BDNF signaling activates CREB in the NAc that can subsequently induce *fosb* transcription (Levine et al., 2005). In contrast, we did not observe any effect of stress on NAc FosB expression at the end of cocaine self-administration. Given that we measured FosB levels 40 days post-stress and that stress-induced increases in NAc FosB protein return to baseline levels after 21 days (Nikulina et al., 2008), this may explain our lack of a stress effect.

However, we did not observe any effect of VTA BDNF overexpression on FosB expression in the dorsal striatum. Given that this region does not receive any direct projections from the VTA (Haber and Knutson, 2010), this may explain why VTA BDNF overexpression increased FosB expression in the NAc, but not in the dorsal striatum. At the same time, we found a stress-induced decrease in dorsal striatum FosB expression. Previous work has shown that FosB transcription is greatly reduced following cessation from cocaine self-administration, and the cocaine exposure-induced accumulated FosB protein disappeared by 21 days of withdrawal (Larson et al., 2010), suggesting potential tolerance effects. Given that the two stressed groups in the present study had the highest amount of cocaine intake during the binge session, cocaine-induced FosB mRNA expression may have been relatively blunted compared to the handled groups, leading to lower FosB accumulation. Together these data suggest that BDNF overexpression-induced increases of FosB is brain region specific and may require direct innervation from the VTA. Further research is needed to directly test this hypothesis.

5. Conclusions

VTA BDNF overexpression in socially-defeated, but not in handled control rats, enhanced cocaine self-administration during prolonged binge access, and BDNF expression in the VTA correlated positively with cocaine intake during the binge test session. Enhanced BDNF expression in the VTA also induced a brain region-specific increase of FosB in the ventral, but not dorsal, striatum. Therefore, variability of BDNF expression in the VTA may

act as an intrinsic risk factor in the propensity to develop compulsive cocaine-taking behavior following stressful life events.

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Highlights

- VTA BDNF overexpression enhanced cocaine binge intake in socially-defeated rats.
- VTA BDNF overexpression in the absence of stress had no effect on cocaine self-administration.
- VTA BDNF overexpression enhanced FosB expression in the nucleus accumbens, but not the dorsal striatum.

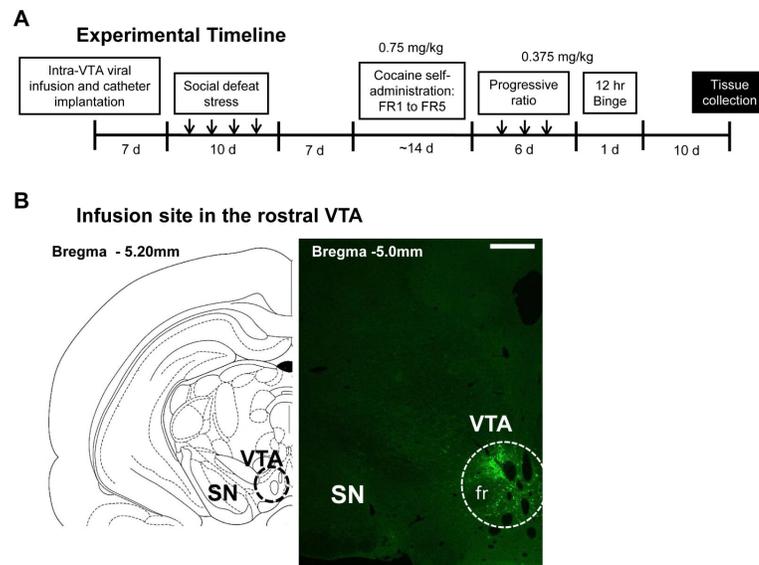


Figure 1. Experimental design schematic and viral infusion site. (A) Timeline of the experimental design. (B) Left panel: Brain atlas hemisection of the rostral VTA at bregma -5.2 mm (Paxinos and Watson, 2007). Dashed circle represents the intended target of viral infusion. Right panel: Fluorescent signal illustrates GFP expression at the infusion site in the VTA of a representative case. Scale bar = $500 \mu\text{m}$. Sections were $20 \mu\text{m}$ thick. (SN: substantia nigra; fr: fasciculus retroflexus).

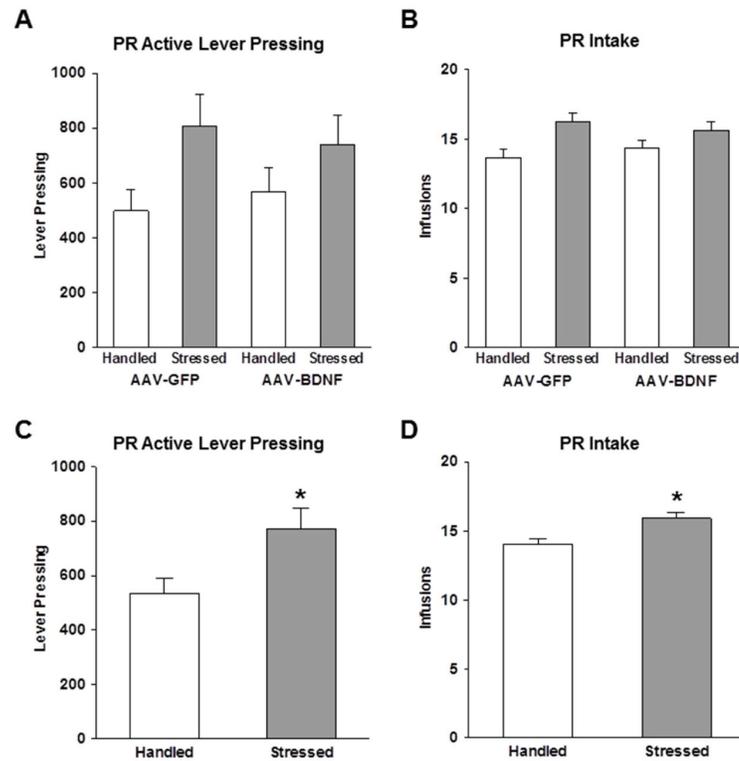


Figure 2. Effect of VTA BDNF overexpression and social defeat stress on cocaine self-administration under a progressive ratio schedule of reinforcement. Average number of active lever presses (A) and cocaine infusions (B; 0.375 mg/kg/infusion, IV) on a progressive ratio (PR) schedule. GFP-Handled: $n = 18$; GFP-Stressed: $n = 11$; BDNF-Handled: $n = 20$; BDNF-Stressed: $n = 13$. Average PR active lever pressing (C) and intake (D) collapsed across virus conditions. * - different from Handled groups, main effect of stress, $p < 0.01$. Handled groups: $n = 38$; Stressed groups: $n = 24$. Error bars indicate SEM.

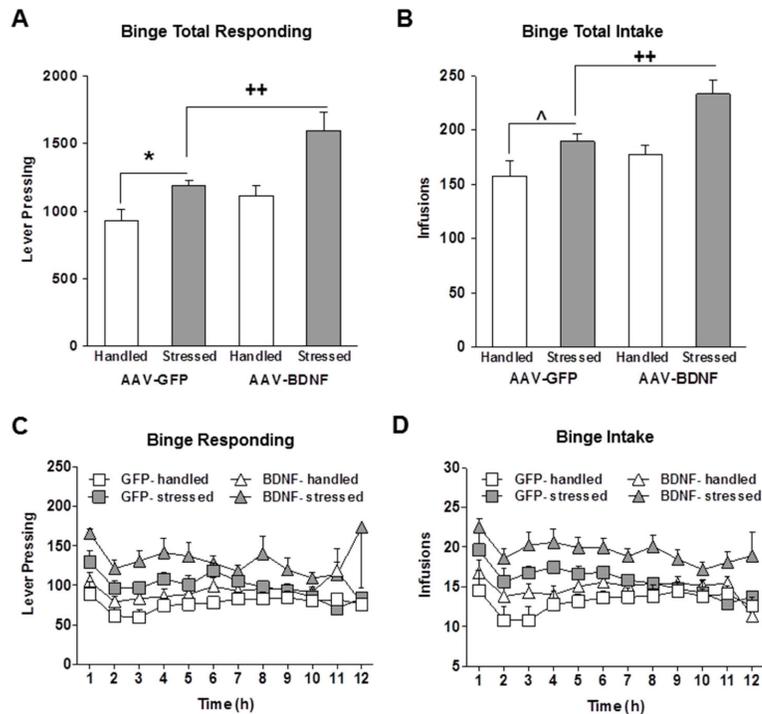


Figure 3. Effects of VTA BDNF overexpression and social defeat stress on binge cocaine self-administration. Total number of active lever presses (A) and cocaine infusions (B; 0.375 mg/kg/infusion, IV) received during the binge session. Significant main effects of stress and virus were present. ^: $P = 0.055$; * - different from GFP-Handled group, planned comparison, $p < 0.05$; ** - different from GFP-Stressed group, planned comparison, $p < 0.01$. Number of active lever presses (C) and cocaine infusions (D) received during the 12-h binge session in 1-h time bins. GFP-Handled: $n = 18$; GFP-Stressed: $n = 11$; BDNF-Handled: $n = 20$; BDNF-Stressed: $n = 13$. Error bars indicate SEM.

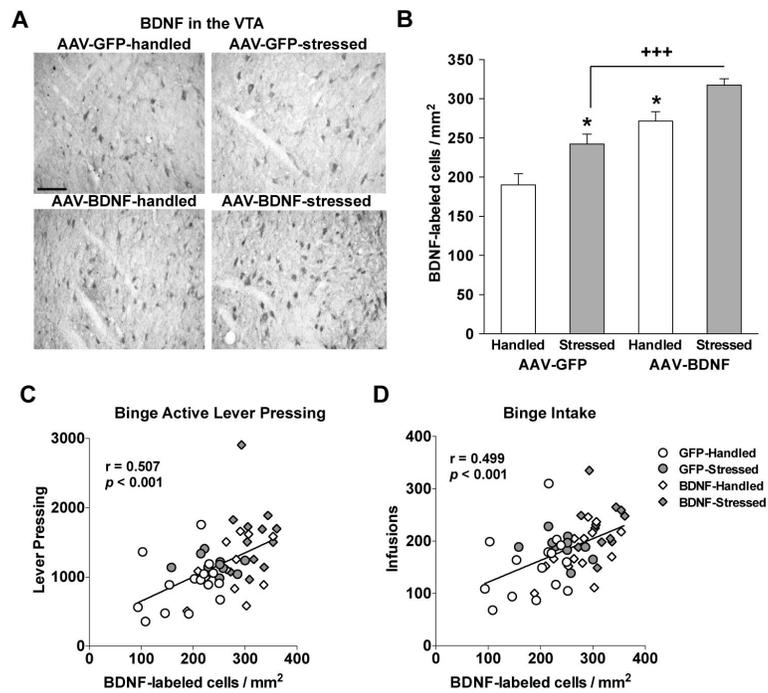


Figure 4.

Positive correlation between VTA BDNF labeling and cocaine self-administration. (A) Representative photomicrographs of BDNF labeling in the VTA of each group; scale bar = 100 μ m. (B) Number of BDNF-labeled cells in the VTA across groups. Significant main effects of stress and virus were observed. * - different from GFP-Handled group, planned comparison, $p < 0.05$; +++ - different from GFP-stressed group, planned comparison, $p < 0.001$. GFP-Handled: $n = 15$; GFP-Stressed: $n = 10$; BDNF-Handled: $n = 15$; BDNF-Stressed: $n = 12$. Errors bars indicate SEM. Scatter plot of the correlation between the amount of VTA BDNF labeling and the number of lever pressing (C) and cocaine infusions (D) during the 12-h binge session, in all groups. Line represents best-fit regression.

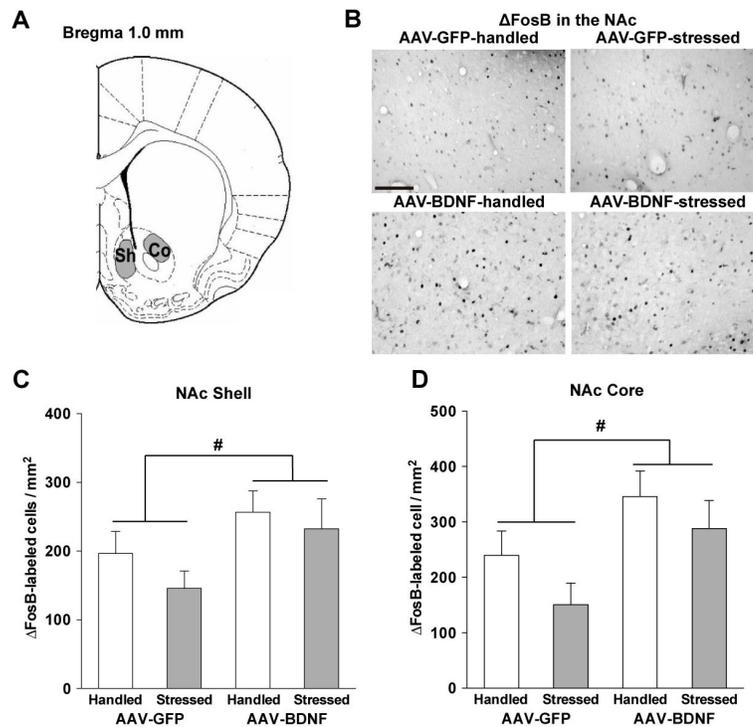


Figure 5.

VTA BDNF overexpression increases FosB expression in the NAc. (A) Brain atlas hemisection showing the assessed regions (shaded) of the NAc shell and core at bregma 1.0 mm (Paxinos and Watson, 2007). (B) Representative photomicrographs of FosB labeling in the NAc shell of each experimental group. Scale bar = 100 μ m. Number of FosB-labeled cells in the NAc shell (C) and core (D) across groups. # - different from GFP groups, main effect of virus, $p < 0.05$. GFP-handled: $n = 12$ (shell), 11 (core); GFP-stressed: $n = 10$ (shell), 9 (core); BDNF-handled: $n = 13$ (shell), 12 (core); BDNF-stressed: $n = 12$ (shell), 11 (core). Error bars indicate SEM.

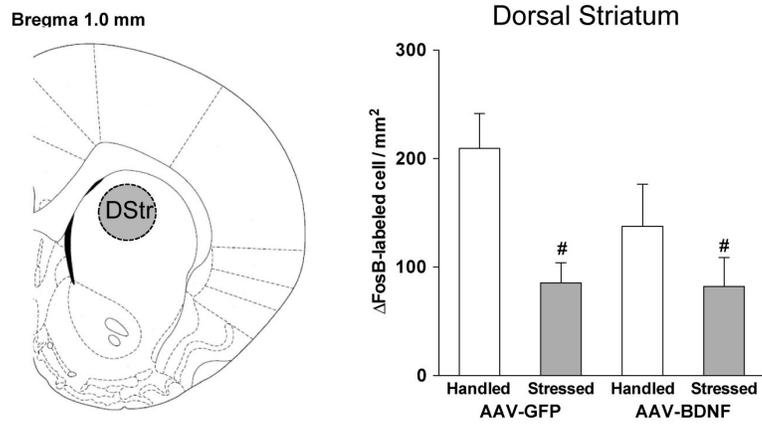


Figure 6.

FosB expression in the dorsal striatum was reduced by stress, but not VTA BDNF overexpression. (A) Brain atlas hemisection showing the assessed regions (shaded) of the dorsal striatum (DStr) at bregma 1.0 mm (Paxinos and Watson, 2007). (B) Number of FosB-labeled cells in the dorsal striatum across groups. # - different from handled groups, main effect of stress, $p < 0.05$. GFP-handled: $n = 12$; GFP-stressed: $n = 9$; BDNF-handled: $n = 12$; BDNF-stressed: $n = 12$. Error bars indicate SEM.

Table 1

Cocaine self-administration measures.

Phase of study	Acquisition	Maintenance	Progressive Ratio	Binge
Groups	Time to Reach Criterion (h) [#]	Session Length (h) ⁺	Inactive Lever Presses	Inactive Lever Presses
GFP-handled	15 ± 3	2.0 ± 0.2	92 ± 19	85 ± 23
GFP-stressed	11 ± 3	1.8 ± 0.2	54 ± 13	50 ± 21
BDNF-handled	10 ± 2	2.1 ± 0.1	80 ± 19	38 ± 17
BDNF-stressed	13 ± 4	1.8 ± 0.2	45 ± 21	82 ± 78

[#] acquisition criterion was defined as time that elapsed from the start of the first session until the rats met the criterion of obtaining 15 infusions during a given session;

⁺ session length was determined by how long it took each rat to obtain 15 infusions in a given session and this was averaged across the five maintenance sessions.