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Brain-derived neurotrophic factor signaling mitigates the impact of acute social stress

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Abstract

Brain-derived neurotrophic factor (BDNF) is known to promote fear learning as well as avoidant behavioral responses to chronic social defeat stress, but, conversely, this peptide can also have antidepressant effects and can reduce depressant-like symptoms such as social avoidance. The purpose of this study was to use a variety of approaches to determine whether BDNF acting on tropomyosin receptor kinase B (TrkB) promotes or prevents avoidant phenotypes in hamsters and mice that have experienced acute social defeat stress. We utilized systemic and brain region-dependent manipulation of BDNF signaling before or immediately following social defeat stress in Syrian hamsters, TrkB^{F616A} knock-in mice, and C57Bl/6J mice and measured the subsequent behavioral response to a novel opponent. Systemic TrkB receptor agonists reduced, and TrkB receptor antagonists enhanced, behavioral responses to social defeat in hamsters and mice. In the neural circuit that we have shown mediates defeat-induced behavioral responses, BDNF in the basolateral amygdala, but not the nucleus accumbens, also reduced social avoidant phenotypes. Conversely, knockdown in the basolateral amygdala of TrkB signaling in TrkB^{F616A} mice enhanced defeat-induced social avoidance. These data demonstrate that systemic administration of BDNF-TrkB drugs at the time of social defeat alters the behavioral response to the defeat stressor. These drugs appear to act, at least in part, in the basolateral amygdala and not the nucleus accumbens. These findings were generalizable to two rodent species with very different social structures and, within mice, to a variety of strains providing converging evidence that BDNF-TrkB signaling reduces anxiety- and depression-like symptoms following short-term social stress.

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Keywords

Social Avoidance; Conditioned Defeat; TrkB; Social Defeat; Basolateral Amygdala; Nucleus Accumbens

1. INTRODUCTION

Social avoidance is a key symptom of a wide variety of neuropsychiatric illnesses^{1–4} including mood and anxiety disorders, post-traumatic stress disorder, and even schizophrenia⁵. These disorders affect millions of individuals^{6–9} and are associated with devastating social, somatic, and economic costs^{8–11}, particularly given that currently-approved treatments often have limited efficacy^{12–15}. These treatments have very limited pharmacodynamic targets, suggesting that novel treatment options aimed at alternative mechanistic pathways are desperately needed. Neurotrophin signaling, particularly that of brain-derived neurotrophic factor (BDNF) at its cognate receptor, tropomyosin receptor kinase B (TrkB), is one such promising alternative target¹⁶.

BDNF has been implicated in a variety of neuropsychiatric disorders^{17–19}. Multiple lines of evidence suggest that impaired BDNF signaling is integral to the pathophysiology of mood disorders and that BDNF is critical for the therapeutic mechanism of antidepressants^{17,19–25}. Chronic treatment with antidepressants, electroconvulsive shock therapy, and exercise all increase BDNF mRNA and protein expression in the hippocampus in humans and rodents^{26–29}, and blocking the activity of BDNF in response to these treatments blocks their therapeutic effect^{25,30–32}. Additionally, a single nucleotide polymorphism in the human BDNF gene (Val66Met, in which methionine is substituted for valine in codon 66) has been associated with increased anxiety and an increased risk of stress-related depression and schizophrenia^{33–36} in humans and increased anxiety- and depression-like phenotypes in mice^{37–40}.

Many studies investigating the relationship between stress and neuropsychiatric disorders have employed social stressors^{41–44}. Social stress is reported to be the most common and salient type of stress experienced by humans and other animals^{45–49}, and exposure to social stress can cause or exacerbate mental illness in humans^{47,50–52} as well as stimulate depression- and anxiety-like behavior in non-human animals^{43,53,54}. A marked social avoidance phenotype is produced in a wide variety of organisms, including hamsters and mice, following exposure to both acute and chronic social defeat stress^{42,55–62}, but the majority of studies have used chronic defeat protocols. Seminal work by Berton et al.⁶³ demonstrated that, in mice, BDNF is required for the development of social avoidance after chronic defeat. This and other studies have established a central role for BDNF-TrkB signaling, particularly in the ventral tegmental pathway to the nucleus accumbens (NAc), in promoting chronic defeat-induced social avoidance and anxiety- and depressive-like behavior in mice^{64–66}. We have previously demonstrated that brief social stress, which also stimulates marked social avoidance and conditioned defeat in Syrian hamsters^{67,68}, increases BDNF mRNA in the hippocampus and in the basolateral and medial amygdala but not in the central amygdala or nucleus accumbens and that microinjection of the non-selective Trk

receptor antagonist k252a into the basolateral amygdala (BLA) reduces these defeat-induced behavioral responses⁶⁹. Together, these data are consistent with the hypothesis that BDNF *promotes* social stress-induced behavioral changes in hamsters as it does in mice. This idea, however, runs counter to the data presented above illustrating that BDNF *prevents or reverses* depression- and anxiety-like responses. Given the interest in BDNF signaling pathways as a possible new target for pharmacological intervention in disorders in which social avoidance is a key symptom, it is important to broaden our understanding of BDNF's effects on stress responding. The purpose of the present project was to employ a comparative approach and a variety of methodologies and behavioral tests using both hamsters and mice to determine whether social defeat-induced behavioral responses are *promoted or prevented* by BDNF-TrkB signaling. We tested whether systemic treatments that alter this signaling pathway are effective in changing behavioral responses to a social stressor and, if so, if these effects could be mimicked by manipulating BDNF-TrkB signaling site specifically in the NAc or BLA.

2. MATERIAL AND METHODS

2.1 Animals

Male Syrian hamsters (*Mesocricetus auratus*) were obtained from Charles Rivers Laboratories (New York, NY or Wilmington, MA) or bred in-house from animals obtained from Charles Rivers. Hamsters were 9–12 weeks old and weighed between 120–160 g at the time of testing. Resident aggressors (RAs) used in defeat training were larger (at least 3–6 months old), singly-housed hamsters proven to reliably attack an intruder. Non-aggressive intruders used during conditioned defeat testing were smaller (7–9 weeks old), group-housed hamsters that did not respond to the resident hamster with overt aggression or submission. All hamsters were housed in polycarbonate cages (23 × 43 × 20 cm) with corncob bedding, cotton nesting material, and wire tops within a colony room on a 14:10 light/dark cycle as is customary in this species to maintain gonadal patency⁷⁰.

Male mice (3–5 months of age) were conditional TrkB^{F616A} (129J/C57Bl/6 hybrid background; TrkB^{F616A} knock-in (KI)) mutants bred in-house from animals originally obtained from Jackson Labs or C57BL/6J mice (Charles Rivers Laboratories). RAs were retired male CD-1 breeders (1–1.5 yrs old) that were individually housed and screened for reliable aggression. Mice were housed in standard cages (19.5 × 13 × 38 cm) with corncob bedding and cotton nesting material within a dedicated colony room on a 12:12 light/dark cycle.

All animals were given *ad libitum* access to food and water, were singly housed 1 week prior to behavioral manipulations, and were handled daily for at least five days to allow habituation to experimenters. One week of single housing is not a significant stressor for hamsters or mice^{71,72}. To minimize circadian variation, all manipulations occurred during the first 3 hr of the dark phase of the daily cycle under dim red illumination. This is the time when rodents are active and exhibit the majority of their agonistic behavior^{73,74} and is thus the most ethologically relevant time window within which to test. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use

Committee and were in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2 Pharmacological Agents

All drug doses were based on those commonly used in the literature. The selective, small molecule TrkB receptor agonist 7,8-Dihydroxyflavone⁷⁵, however, see also⁷⁶ (7,8-DHF) (TCI America; Montgomeryville, PA) was administered intraperitoneally (IP) either 1 hr before (acquisition study) or immediately after (consolidation study) defeat training in hamsters. Hamsters received one of three doses (2.5, 5.0, or 10mg/kg) of 7,8-DHF dissolved in 40% Dimethylsulfoxide (DMSO)/60% saline or vehicle (40% DMSO/60% saline), and mice received 5mg/kg 7,8-DHF or vehicle administered 1 hr prior to social defeat training^{77,78}. The selective TrkB receptor antagonist ANA-12 ([N2–2-2-Oxoazepan-3-yl amino] carbonyl phenyl benzo (b) thiophene- 2-carboxamide) (Sigma; St. Louis, MO)⁷⁹ was also administered IP (0.0 mg/kg, 0.5 mg/kg or 1.0 mg/kg in 100% DMSO). BDNF (rhBDNF; Sigma; St. Louis, MO) was administered site-specifically via microinjection (0.2ng, 0.4ng or 0.8ng/200nl physiological saline) immediately before social defeat training.

Reversible TrkB signaling blockade in mutant TrkB^{BF616A} KI mice was induced using site-specific, bilateral infusions of 1-NM-PP1 (250 nl/site of 0.1 nmol 1-NM-PP1 in 4% DMSO/2% Tween-20; Millipore Sigma, Burlington, MA)^{80,81}. TrkB^{BF616A} KI mice given vehicle functioned as wildtype controls and untreated TrkB^{BF616A} KI mice, which have fully functional TrkB receptors, served as subjects for peripheral 7,8-DHF injections. C57Bl/6J were used to test effects of BDNF in the BLA.

2.3 Cannulations, Microinjection Procedures, and Histological Verification of Injections

For central drug administration, subjects were implanted with bilateral guide cannulae targeting the BLA or NAc. Skulls were leveled before implantation and final stereotaxic coordinates were measured from Bregma: BLA (hamsters: +0.0AP, $\pm$ 4.0ML, –6.2DV; mice: –1.6AP, $\pm$ 3.3ML, and –4.9DV), NAc (hamsters: +3.2AP, $\pm$ 3.1ML, –4.0DV at a 20° angle toward the midline); mice: +1.2AP, $\pm$ 2.3ML, –4.7DV at a 20° angle toward the midline). Anesthesia was induced with 5% isoflurane and then maintained at 2–3% isoflurane during surgery. Dummy stylets were placed in cannulae and these were removed and replaced daily during handling to ensure patency and to habituate subjects to handling and injection procedures. Microinjections were completed using an infusion pump (Harvard Apparatus, Holliston, MA) and a Hamilton syringe that was connected to the injection needle by 50-gauge polyethylene tubing. To minimize damage to the injection site, shorter, 26-gauge guide cannula were used with a longer, 33-gauge injection needle that projected 1.2 mm beyond the guide cannula to provide the final depth. Microinjections were administered over 1–2 min, and the injection needle was left in the cannula guide for 1 min after the injection to ensure diffusion of the drug from the needle tip. Successful injections were inferred if solution flowed easily from the needle before and after microinjection and if a small air bubble placed between the drug and saline solution in the tubing moved during the microinjection.

At the conclusion of experiments with centrally administered drugs, subjects were deeply anesthetized, decapitated and infused with 200nl of India ink per cannula to verify needle placements. Brains were then removed from the skull and flash frozen in isopentane and dry ice and stored at -80°C until sectioning on a cryostat (Leica CM 3050 S). Mounted sections were stained with Neutral red, cover slipped with DPX mountant, and examined using a light microscope for ink in the target region. Only animals with ink injections within 0.3mm of the target region, as determined by observers blind to experimental group, were included in the final analysis.

2.4 Social Defeat Training

For hamsters, social defeat training was performed as described previously⁵⁵. Briefly, defeats occurred in the home cage of an RA for a duration of either 5 or 15 min. We used “suboptimal”, 5 min defeats when we expected a treatment to enhance defeat-induced behaviors to avoid ceiling effects in conditioned defeat during testing, and we used 15 min defeats when we expected the opposite to avoid floor effects in conditioned defeat. For mice, defeat training occurred in the home cage of a CD1 aggressor and lasted 5 min after the initial defeat of the subject, characterized by the RA attacking the subject and the subject exhibiting subsequent submissive behavior and flight. All animals were monitored during the defeat experience to ensure that no physical injuries occurred. During training, no defeat controls were placed into an empty RA/CD1 cage for the same amount of time as defeated animals to control for handling and exposure to a novel cage with social odors. Following these manipulations, all subjects were returned to their home cages. When drug infusions were given prior to defeat training, behavior of the aggressors and subjects was digitally recorded and scored, as described below, to ensure that there were no differences in the amount of aggression emitted by the aggressor or submission exhibited by the subject as a factor of drug treatment.

2.5 Behavioral Testing, Scoring, and Analysis

Behavioral testing occurred 24 hrs after defeat training and was digitally recorded via CCD camera for later scoring by observers blinded to condition using Noldus Observer (v. 7.0–11.5; Leesburg, VA, USA). Hamsters were tested for conditioned defeat, defined as a lack of territorial aggression and a display of submissive behavior, social avoidance, and flight, by placing a non-aggressive intruder into the subject’s home cage for 5 min^{43,55}. During conditioned defeat testing in hamsters, the duration of all behaviors emitted was measured and grouped in the following categories: (1) Social (i.e., greet, sniff, allogroom) (2) Non-social (i.e., locomotion, autogroom) (3) Submissive/Defensive/Avoidant (i.e., upright and side submissive posture, tail lift, flee) and (4) Aggressive (i.e., upright and side attack, bite, chase), as described in detail in Albers, Huhman, & Meisel⁸². Conditioned defeat testing with an intruder was used instead of simply testing social avoidance in hamsters because this procedure allows us to examine whether any of the treatments effect aggression and other behaviors, and thus potentially allows a richer examination of the behavioral response to defeat than is obtained simply by measuring social avoidance.

Because mice will neither attack nor avoid a conspecific introduced into their home cage, this species is usually tested for defeat-induced social avoidance of a caged opponent⁴². We

tested mice for defeat-induced social avoidance of a caged, unfamiliar CD1 aggressor (duration 5 min) in a novel arena (polycarbonate cage: 24 × 33 × 20 cm). The CD1 aggressor was placed in a plastic mesh cage (6.75 × 6.75 × 3.5 cm) on one end of the arena to allow visual, olfactory, and auditory communication without physical interaction as is common in the mouse social defeat literature. The subject was allowed to freely ambulate around the testing arena and an observer blinded to treatment group scored the duration of time (sec) spent in the far half of the arena from the caged opponent (our operational definition of social avoidance) and in the social interaction zone immediately surrounding the caged opponent. Observers also scored frequency of risk assessments, indicated by a characteristic flat-backed, stretch-attend posture towards, as well as flees away from, the caged opponent^{68,83–85}. We did not calculate a social interaction ratio but instead directly measured frequencies and/or durations of the behaviors emitted because we were expressly interested in the effect of the BDNF/TrkB manipulations on avoidance-like behavior in the population as a whole and were not interested in dividing animals into “resilient” and “susceptible” groups.

Statistical analyses were performed with IBM SPSS software (v. 20.0.0–23.0.0) using *a priori* t-tests or analysis of variance (ANOVA) followed by Tukey’s tests, where appropriate. Effect sizes were calculated with Cohen’s d for t-tests or eta-squared for ANOVAs.

3. RESULTS

3.1 Behavioral Responses to Social Defeat in Hamsters and Mice

Because there were 5 and 15 min defeats in hamsters, the data are shown as percent of defeat-vehicle control for consistency and ease of comparison among experiments. Only a subset of the behavioral data are presented in the main results and figures, but the complete behavioral data for all behaviors scored during testing, as well as statistical comparisons and effect sizes for the raw data in seconds, are shown in the Supplemental Results and Figures (S1–S4). It is also important to note that no animals were included in the analysis if they sustained any bite that broke the skin during defeat training; thus, all of these animals have experienced primarily psychological stress and no wounding. In this study, only one animal (a hamster) was removed from the analysis due to a bite wound.

There was little to no aggression displayed by any defeated hamsters or mice in this study and no significant effect of treatment on aggression in any of the experiments. The data for no defeat vehicle and no defeat drug groups, when included, are also shown in the Supplemental Figures. To reduce the number of animals required, no defeat controls were not included in every experiment.

3.2 Systemic Administration of a TrkB Receptor Agonist Reduces Conditioned Defeat in Hamsters and Defeat-Induced Social Avoidance in Mice.

We first tested whether a specific, non-peptide TrkB receptor agonist administered systemically alters behavioral responses to social defeat in hamsters and mice. We initially gave one of three doses of 7,8-DHF before defeat training in hamsters and found that there was a significant main effect of drug on submissive behavior emitted during conditioned

defeat testing (Fig 1a–b; Submission: $F(3,39)=2.946$, $p=0.045$; $\eta^2=0.185$). Tukey's *post-hoc* tests revealed that hamsters receiving the highest dose of 7,8-DHF before defeat exhibited significantly less submissive behavior than did hamsters that received vehicle ($p=0.032$; Cohen's $d=1.213$). If hamsters were given 7,8-DHF immediately following defeat training, there was still a significant effect of drug on submissive behavior (Fig 1c–d; $t_{(19)}=2.089$, $p=0.050$; Cohen's $d=0.9$). Effects of 7,8-DHF on all scored categories of behavior are shown in seconds in Supplemental Figure 1.

Next, we tested whether 7,8-DHF would also reduce the behavioral response to defeat in mice (Fig 1e–f). In mice that received 7,8-DHF we observed a significant reduction in avoidance behavior (Fig 1g; $t_{(26)}=-2.379$, $p=0.025$; Cohen's $d=0.905$) as well as a significant increase in social interaction (Fig 1h; ($t_{(26)}=-2.243$, $p=0.034$; Cohen's $d=0.86$)). There was a trend toward decreased risk assessments (Fig 1i; ($t_{(26)}=1.139$, $p=0.063$; Cohen's $d=0.74$)).

3.3 Systemic Administration of a TrkB Receptor Antagonist Enhances Conditioned Defeat in Hamsters

We next tested whether blockade of TrkB receptor signaling following systemic administration of ANA-12 would enhance behavioral responses to social defeat in hamsters subjected to a 5 min defeat (Fig 2a). Indeed, hamsters given ANA-12 immediately following social defeat training displayed more submissive behavior during conditioned defeat testing than did defeated hamsters receiving vehicle (Fig 2b; $t_{(10,583)}=-2.254$, $p=0.046$; Cohen's $d=1.000$). Effects of ANA-12 on all scored categories of behavior are shown in seconds in Supplemental Figure 2.

3.4 BDNF in the NAc Does Not Alter Behavior Following Acute Defeat in Hamsters or Mice

BDNF in the pathway from the ventral tegmental area (VTA) to the NAc has been shown to be necessary for the behavioral changes that are observed in mice following chronic social defeat stress^{63,64}. We tested whether microinjection of BDNF in the NAc before a 5 min defeat would enhance conditioned defeat in hamsters (Figs 3a–c) or social avoidance in mice (Fig 3d–f). There was no main effect of BDNF on submissive behavior in hamsters (Fig 3b; $F_{(2,24)}=0.509$, $p=0.607$; $\eta^2=0.041$) or on social avoidance in mice (Fig 3e; $F_{(1,21)}=0.023$, $p=0.880$; $\eta^2=0.001$). Effects of BDNF on all scored categories of behavior are shown in seconds in Supplemental Figure 3.

3.5 BDNF-TrkB Signaling in the BLA Alters Defeat-Induced Behavior in Hamsters and Mice

Finally, we tested whether altering BDNF-TrkB signaling in the BLA before defeat training changes defeat-induced behavioral responses. Hamsters microinjected bilaterally with BDNF in the BLA exhibited significantly less submission than did vehicle controls (Fig 4a–c $t_{(19,756)}=-2.278$, $p=0.034$; Cohen's $d=0.784$). There was no effect of BDNF on any other behavioral category (see Supplemental Figure 4).

In mice, we tested whether TrkB signaling *blockade* in TrkB^{F616A} KI mice promotes defeat-induced social avoidance. Indeed, mice infused with 1-NM-PP1 1h before defeat training exhibited significantly more social avoidance during testing with a caged opponent than did vehicle-infused mice (Fig 4d-f; $t_{(17)}=-2.518$, $p=0.022$; Cohen's $d=1.145$).

4. DISCUSSION

The present project used a novel comparative, multidimensional approach to demonstrate that both peripheral and brain region-specific manipulation of BDNF signaling alters behavioral responses to acute social defeat stress and that these effects are similar in hamsters, which are territorial and aggressive, and mice, which are more social and less aggressive. Significantly, despite considerable evidence from mouse chronic defeat studies that BDNF signaling *promotes* social avoidance, here we demonstrate in both hamsters and in mice that activating BDNF signaling during or after a brief social defeat stressor *reduces* subsequent social avoidance and, conversely, that inhibiting BDNF signaling enhances this behavioral response. These effects appear to be mediated in hamsters and mice via the action of BDNF on TrkB receptors and are generalizable to two different behavioral tests of social avoidance. The data also suggest that TrkB agonists, or pharmaceutical approaches that result in TrkB activation, might have prophylactic effects if administered before or soon after exposure to a stressor.

These data also demonstrate that measuring conditioned defeat in hamsters that are tested with a freely moving, non-aggressive intruder produces comparable data to that generated in mice using social avoidance of a caged opponent as the dependent measure. Although we have shown that hamsters, like mice, display social avoidance to caged opponents^{68,85-87}, conditioned defeat testing in hamsters adds complementary data in that it is possible to measure not only submission and social avoidance, but also to determine if treatments have any effect on aggression. Importantly, we observed no changes in aggressive behavior following any of the treatments used in this study.

The majority of the existing literature demonstrating that BDNF promotes behavioral responses to social defeat has been generated using pharmacologic, genetic, or optogenetic manipulations of BDNF signaling primarily within the pathway from the VTA to the NAc⁶³⁻⁶⁵. Because it is important from a translational perspective to understand how global alteration of BDNF signaling alters behavior, we began with peripheral administration of specific TrkB receptor agonists and antagonists. Instead of promoting behavioral responses to defeat, however, we demonstrated that a systemic TrkB receptor agonist given either before or immediately after the initial defeat reduced the behavioral response to defeat in both hamsters and mice and that TrkB receptor antagonism or knockdown enhanced this response. One possibility to account for the finding that these drugs have the opposite effect from that observed in the VTA-NAc is that systemically administered BDNF-active drugs primarily affect a brain region or regions other than the VTA-NAc. There is a precedent for the idea that BDNF signaling acts in a brain region-specific manner to either promote or prevent behavioral responses to social defeat¹⁶. In rats that fail to respond to social defeat with an avoidant phenotype, BDNF mRNA and protein are higher in the hippocampus and blocking BDNF signaling there promotes social avoidance; conversely, 7,8-DHF reduces

social avoidance in rats that are susceptible to defeat⁸⁸. Similarly, in mice that are susceptible to chronic social defeat, BDNF, TrkB, and phosphorylated TrkB proteins are reduced in prefrontal cortex and hippocampus following chronic social defeat, and antidepressant treatment significantly attenuates this reduction as well as reverses the social avoidance phenotype⁸⁹. Further, the antidepressant-induced reduction in social avoidance is blocked by systemic ANA-12 indicating that the effect is dependent on TrkB signaling⁸⁹. Finally, BDNF has an antidepressant-like effect when microinjected into the hippocampus^{17,90}, but pro-depressant effects are observed when BDNF is given in the VTA-NAc pathway (⁶⁶; however, see also⁹¹). Together, the data strongly support the contention that BDNF signaling in brain promotes and prevents depressive-like, social avoidance phenotypes in a region-dependent manner but also that systemic treatments that enhance BDNF signaling reduce stress-induced behavioral responses.

In the brain, we chose to focus on the BLA in addition to the NAc because we have previously demonstrated that synaptic plasticity in the BLA is necessary for the behavioral response to brief social defeat in hamsters^{92–94}. We have also demonstrated that the NAc is part of the neural circuit controlling the acquisition and expression of conditioned defeat in hamsters^{95,96}. Here, we found that manipulation of BDNF signaling in the BLA, but not in the NAc, altered behavioral responses to defeat in hamsters and mice. Notably, the behavioral changes following BLA manipulations (BDNF microinjections in hamsters or TrkB inactivation in TrkB616A KI mice) were consistent with the effects of the systemic treatments, suggesting that these peripheral treatments may be acting, at least in part, in the BLA. The existing literature also suggests that the hippocampus is a likely target for antidepressant (e.g., social avoidance-reducing) effects of BDNF-active treatments^{88,89,97}, but this possibility remains to be tested in our models. There are several reasons why we may have not observed an effect of our treatments in the NAc in either hamsters or mice. The most obvious is that the literature demonstrating avoidance-promoting effects of BDNF in the VTA-NAc pathway have been established following a much more severe and prolonged defeat stressor (chronic social defeat stress, see^{42,63–65,98,99}) than was used in our study. It is possible that BDNF in the amygdala is a critical modulator of the behavioral response to brief, relatively mild, or episodic stressors or in the early phases of responding to a social stressor, while BDNF in the NAc plays a critical role in promoting avoidant phenotypes resulting from more robust or chronic stressors. This shift would be consistent with the finding in rats that episodic social stress increases the number of BDNF-immunoreactive cells while prolonged social stress decreases BDNF immunoreactivity in the VTA¹⁰⁰.

Other important factors could contribute to differences among studies in the area of social defeat, and more care should be taken in the field to standardize these procedures and to consider both their ethological relevance and their potential influence on the resulting data. Many of the studies discussed herein have divided defeated subjects into two groups based on their behavioral responses, one that displays defeat-induced social avoidance (often called “susceptible” or “vulnerable”) and another that does not (called “resilient”, “resistant”, or “unsusceptible”). Studies of BDNF effects following chronic social defeat stress have often used only the susceptible animals as subjects. The division of subjects into susceptible and resistant sub-groups is clearly a powerful way to examine the mechanisms

that underlie individual differences in stress responding but could also affect the conclusions that are reached following pharmacological manipulations. By contrast, we did not divide subjects into responders versus non-responders; all defeated animals were included in testing and analyses, potentially enhancing the translational value of the present findings. Another important factor that is often ignored is the effect of lighting and circadian variability. In studies that give information about when testing was done and under what lighting conditions, the vast majority tested animals in bright light during the inactive (light) phase of the daily cycle. By contrast, all of our defeat training and testing is done under dim red illumination during the first three hours of the dark phase of the daily cycle, the time when the animals are most active and when they normally emit almost all of their agonistic behavior^{73,74}. It is well documented that stress responding can vary dramatically across the daily light-dark cycle (for a review, see¹⁰¹), so differences among labs in when defeat and testing occur could account for at least some of the discrepancies. We argue that ethologically-relevant behavioral procedures should be done during the circadian window when subjects would normally be active and producing the relevant behaviors.

It must be recognized that our current findings appear inconsistent with our previous report that social stress enhances BDNF mRNA and that the k252a blocks the formation of conditioned defeat in hamsters⁶⁹. It should be noted, however, that BDNF mRNA was significantly elevated above control levels in the BLA of both dominant as well as subordinate hamsters, indicating that increases in BDNF mRNA are not unique to defeated animals. BDNF mRNA was unchanged in the NAc, again suggesting that BDNF in the NAc may not regulate behavioral responses to acute social defeat. It is also the case that an increase in mRNA may not translate to more protein or signaling¹⁰². Furthermore, k252a, which is often erroneously declared a TrkB receptor antagonist, is actually a non-selective protein kinase inhibitor that blocks the action of an array of trophic factor signaling at their respective receptors¹⁰³. Thus, the current approach using newer, selective, small molecule TrkB-active drugs is a more conclusive test of the putative role of BDNF-TrkB signaling in the establishment of defeat-induced social avoidance.

The current data also run counter to the findings of Dulka et al.⁶¹, who demonstrated in mice that BLA microinjections of α_2 -antiplasmin, a drug that blocks enzymatic conversion of the large precursor protein, proBDNF, to the mature form of BDNF (mBDNF), significantly reduced subsequent social avoidance after a brief defeat. They concluded that proteolytic cleavage of proBDNF into mBDNF is necessary for conditioned defeat learning to occur. The authors acknowledge, however, that it is possible that a buildup of proBDNF, which acts via the p75^{NTR} receptor to exert opposite effects of mBDNF, and not a reduction in mBDNF accounts for the treatment-induced decrease in social avoidance. Future studies should determine what actions are mediated by BDNF and what might be the role of proBDNF-p75 in these effects.

Another possibility that needs to be explored is whether the balance of activity among the components of the neural circuit that controls behavioral responses to social defeat is altered when a stressor shifts from acute to chronic in duration. Similarly, it will be important to determine whether BDNF signaling prevents behavioral stress responses initially or

following mild stress, but then begins to promote these responses when the stressor becomes chronic or more severe.

5. CONCLUSIONS

In sum, we have demonstrated using two different rodent models of social stress and two different measures of defeat-induced behavior that stimulating BDNF-TrkB signaling reduces and that inhibiting TrkB signaling enhances behavioral responses to a brief social stressor. This effect was observed with systemic drug treatments as well as with brain region-specific manipulations. We also demonstrated that the behavioral effect observed after systemic manipulation of BDNF-TrkB signaling is likely mediated at least in part in the BLA and not the NAc. Finally, the fact that BDNF-TrkB manipulations altered behavior similarly in a species that is considered aggressive and relatively non-social and another species that is considered social suggests that this effect may have translational relevance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Enhancing BDNF-TrkB signaling reduces behavioral responses to acute social defeat.
- Reducing BDNF-TrkB signaling enhances behavioral responses to acute social defeat.
- BDNF-TrkB treatments similarly alter defeat-induced behavior in hamsters and mice.
- Systemic and brain region-specific manipulations of BDNF signaling are effective.

Figure 1.

Systemic administration of a tropomyosin receptor kinase B (TrkB) receptor agonist reduces social defeat-induced behavioral changes in hamsters and mice. All values are shown as percent vehicle control. **(A)** Schematic of the experimental protocol for the hamster acquisition experiment. **(B)** There was a main effect of intraperitoneal (IP) 7,8-dihydroxyflavone (7,8-DHF) given immediately before a 15 min social defeat ($F(3,39)=2.946$, $p=0.045$; $\eta^2=0.19$). Hamsters given 10mg/kg of 7–8,DHF displayed significantly less submission during testing 24 hr later than did hamsters given vehicle (Tukey's *post hoc*: * $p=0.032$; Cohen's $d=1.21$). **(C)** Schematic of the experimental protocol for the hamster consolidation experiment. **(D)** 7,8-DHF (10 mg/kg) given IP immediately after a 15 min social defeat significantly reduced defeat-induced submission during testing compared to vehicle controls ($t(12.052)=2.959$, * $p=0.05$; Cohen's $d=0.90$). **(E)** Schematic of the experimental protocol for the mouse acquisition experiment. **(F)** Schematic of mouse avoidance testing arena. Seconds spent in "Far" was operationally defined as avoidance. Seconds spent within 8 cm of the caged opponent was operationally defined as social interaction. Animals were determined to be within a zone when both forepaws were within that zone. **(G)** Mice given IP 7,8-DHF immediately before social defeat displayed significantly less social avoidance during subsequent testing than did mice given vehicle ($t(26)=2.379$, * $p=0.025$; Cohen's $d=0.91$). **(H)** Mice given IP 7,8-DHF immediately before social defeat displayed significantly more social interaction than did mice given vehicle/defeat ($t(26)=-2.243$, * $p=0.034$; Cohen's $d=0.86$). **(I)** There was a trend for mice given IP 7,8-DHF immediately before social defeat to display fewer risk assessments than did mice given vehicle/defeat ($t(26)=1.139$, $p=0.063$; Cohen's $d=0.74$).

Figure 2.

Systemic administration of a tropomyosin receptor kinase B (TrkB) receptor antagonist enhances conditioned defeat in hamsters. All values are shown as percent vehicle control. **(A)** Schematic of the experimental protocol for the ANA-12 ([N2-2-2-Oxoazepan-3-yl amino] carbonyl phenyl benzo (b) thiophene- 2-carboxamide) consolidation experiment. ANA-12 was given intraperitoneally (IP) only after defeat training because there was a significant effect of the DMSO vehicle on the aggressive behavior produced by resident aggressors towards the experimental animals during defeat training (data not shown). **(B)** Hamsters administered the TrkB receptor antagonist ANA-12 (1.0 mg/kg) immediately after social defeat displayed significantly more submissive behavior when tested with a non-aggressive intruder 24 hr later than did defeated hamsters receiving only vehicle ($t(10.583)=2.254$, $p=0.046$; Cohen's $d=1$).

Figure 3.

Brain derived neurotrophic factor (BDNF) in the nucleus accumbens (NAc) does not alter behavioral responses to acute defeat in hamsters or in C57BL/6J mice. All values are shown as percent vehicle control. **(A)** Schematic of the experimental protocol for hamster BDNF in the NAc. **(B)** BDNF in the NAc did not alter conditioned defeat, even when the dose was doubled ($F(2,24)=0.509$, $p=0.607$; $\eta^2=0.041$). **(C)** Schematic for microinjection sites for data shown in panel (B). Orange shading represents the injection sites for one or more animals. Illustrations were modified from⁹⁹. Final stereotaxic coordinates for hamster NAc were +3.2AP, ± 3.1 ML, and -4.0 DV. To minimize damage to the injected brain regions, our guide cannulae ended 1.2 mm above the targeted site, and an injection needle that projected 1.2 mm from the bottom of the guide cannulae was used to reach the final depth. **(D)** Schematic of the experimental protocol for BDNF in the NAc of C57BL/6J mice. **(E)** BDNF in the NAc did not significantly alter social avoidance in mice that underwent acute social defeat ($t(14)=0.452$, $*p=0.658$; Cohen's $d=0.23$). **(F)** Schematic of microinjection sites for data shown in panel (E). Orange shading represents the injection sites for one or more animals. Illustrations were modified from¹⁰⁰. Final stereotaxic coordinates for mouse NAc microinjections were -1.2 AP, ± 2.3 ML, and -4.7 DV with cannulae angled in toward the midline at 20° .

Figure 4.

Manipulation of tropomyosin receptor kinase B (TrkB) signaling within the basolateral amygdala (BLA) alters responses to social defeat. All values are shown as percent vehicle control. **(A)** Schematic of the experimental protocol for hamster brain-derived neurotrophic factor (BDNF) in the BLA. **(B)** Hamsters implanted with bilateral guide cannulae and microinfused with BDNF (0.4ng/200nl) into the BLA prior to social defeat displayed significantly less submissive behavior during non-aggressive intruder testing than did hamsters receiving only vehicle ($t(19.756)=2.278$, $*p=0.034$; Cohen's $d=0.78$). Group n 's are larger because two experimenters separately replicated this experiment. **(C)** Schematic for injection sites for data shown in panel (B). Orange shading represents the injection sites for one or more animals. For all experiments with central administration, ink injections in each cannula were used to verify needle placement, and only animals with ink injections within 0.3mm of the target region were included in the final analysis. Illustrations were modified from¹⁰³. Final stereotaxic coordinates for hamster BLA were +0.0AP, ± 4.0 ML, and -6.0 DV, and skulls were leveled before implantation. To minimize damage to the injected brain regions, our guide cannulae ended 1.2 mm above the targeted site, and an injection needle that projected 1.2 mm from the bottom of the guide cannulae was used to reach the final depth. **(D)** Schematic of the experimental protocol for mouse 1-NM-PP1 in the BLA. **(E)** Reducing TrkB signaling within the BLA of TrkB^{BF616A} KI mice with 1-NM-PP1 resulted in significantly increased social avoidance during testing 24hr later when compared to mice that received vehicle ($t(17)=2.518$, $*p=0.022$; Cohen's $d=1.15$). **(F)** Schematic of microinjection sites for the TrkB^{BF616A} KI mice shown in panel (E). Orange shading represents the injection sites for one or more animals. Illustrations were modified from¹⁰⁴. Final stereotaxic coordinates for mouse BLA microinjections were -1.6 AP, ± 3.3 ML, and -4.9 DV.