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DIFFERENTIAL BDNF EXPRESSION IN LIMBIC BRAIN REGIONS FOLLOWING SOCIAL DEFEAT OR TERRITORIAL AGGRESSION

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Abstract

Syrian hamsters readily form dominant-subordinate relationships under laboratory conditions. Winning or losing in agonistic encounters can have striking, long-term effects on social behavior, but the mechanisms underlying this experience-induced behavioral plasticity are unclear. The present study tested the hypothesis that changes in brain-derived neurotrophic factor (BDNF) may at least in part mediate this plasticity. Male hamsters were paired for 15-min using a resident-intruder model, and individuals were identified as winners or losers on the basis of their behavior. BDNF was examined with *in situ* hybridization 2 hours after treatment during the consolidation period of emotional learning. Losing animals had significantly more BDNF mRNA in the basolateral (BLA) and medial (MeA) nuclei of the amygdala when compared to winning animals as well as novel cage and home cage controls. Interestingly, winning animals had significantly more BDNF mRNA in the dentate gyrus of the dorsal hippocampus (DHPC DG) than did losing animals, novel and home cage controls. No conflict-related changes in BDNF mRNA were observed in several other regions including the bed nucleus of the stria terminalis and central amygdala. Next, we demonstrated that K252a, a Trk receptor antagonist, significantly reduced the acquisition of conditioned defeat when administered within the BLA. These data support a model in which BDNF-mediated plasticity within the BLA supports learning of submission or subordinate social status in losing animals, whereas BDNF-mediated plasticity within the hippocampus may instantiate aspects of winning such as control of a territory in dominant animals.

Keywords

stress; plasticity; basolateral amygdala; hippocampus; submission

Introduction

Virtually all organisms are exposed to stress. An understanding of the behavioral and physiological responses to stress is important because many psychopathologies (i.e., depression and post-traumatic stress disorder) are stress-related. Stress physiology can be studied in the laboratory with a variety of models including foot-shock, forced swim, and prolonged periods of restraint. These stressors are reliable, but they may have little relevance

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to the daily lives of most organisms. Social stress reliably affects the brain and behavior and is experienced by a wide variety of organisms. Laboratory models of social stress include the visible burrow system, over-crowding, and social defeat. Social stress models are particularly useful because they are considered ethologically relevant and because much, if not most, of the stress encountered by humans occurs in a social context (Plotsky et al., 1998; Bjorkqvist, 2001; Buwalda et al., 2005).

Social stress occurs largely in the form of social conflict. In non-humans, such conflicts generally occur between two male conspecifics that are competing for access to resources such as food or territory as well as for potential mates. At the conclusion of the conflict, a “winner” (i.e., dominant) and “loser” (i.e., subordinate) are readily identifiable. Dominant and subordinate animals often display disparate physiological responses following social conflict with subordinate animals demonstrating increased activation of the hypothalamic-pituitary adrenal (HPA) axis when compared with dominant animals (Huhman et al., 1990; Huhman et al., 1991; Huhman et al., 1992; Blanchard et al., 1995; Spencer et al., 1996). Other physiological changes that occur in subordinate animals include increases in autonomic system activity (Brain, 1980) heart rate, blood pressure, core body temperature (Meehan et al., 1995; Tornatzky et al., 1993), and adiposity (Foster et al., 2006). Chronic social defeat also suppresses immune function as measured by lymphatic organ size (Blanchard et al., 1995) and humoral immune function (Bohus et al., 1993; Fleshner et al., 1989; Jasnow et al., 2001).

Striking behavioral changes are often observed in subordinate animals following an agonistic encounter. For example, subordinates may display decreases in overall locomotor activity and social contact and increased social avoidance (Blanchard and Blanchard, 1989; Meerlo et al., 1996; Meerlo et al., 1997; Shively, 1998; Berton et al., 2006; Duclot et al., 2011; Razzoli et al., 2011a). Chronic social defeat also alters food and water consumption (Meerlo et al., 1996; Foster et al., 2006; Duclot et al., 2011; Razzoli et al., 2011a) and disrupts reproductive behaviors (Blanchard & Blanchard, 1989). Finally, subordinate animals display increased submissive and defensive behaviors when in the presence of a conspecific, and they often fail to defend their own territory (Meerlo et al., 1996; Potegal et al., 1993; van de Poll et al., 1982). An example of this occurs in male Syrian hamsters. After being briefly exposed to a larger, more aggressive counterpart, defeated male hamsters subsequently fail to display normal territorial aggression even when a non-aggressive, smaller hamster is introduced into the home cage of the defeated animal. This behavioral change is termed conditioned defeat (Potegal et al., 1993) and is maintained for at least one month in many animals (Huhman et al., 2003).

Because social stress is hypothesized to be important in the etiology of depression and a variety of anxiety disorders (Nemeroff, 1998; Arborelius et al., 1999; Patten, 1999), it is critical to study the neurobiological processes involved in the behavioral plasticity following social stress. An attractive candidate for mediating social stress-induced behavioral changes is brain-derived neurotrophic factor (BDNF).

BDNF is a neurotrophin that belongs to the nerve growth factor family of peptides. The neurotrophins (including nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin 4/5, acidic- fibroblast growth factor (FGF), basic FGF, and BDNF) are well-known for their role in the development and maintenance of the nervous system (Barde et al., 1982; Leibrock et al., 1989). BDNF and its receptor, TrkB, are critical for synaptic and behavioral plasticity (Lo, 1995; Thoenen, 1995; McAllister et al., 1999). BDNF is rapidly upregulated in the hippocampus following spatial and contextual learning (Hall et al., 2000; Mizuno et al., 2000) and in the amygdala following fear conditioning (Rattiner et al., 2004a)

BDNF mRNA expression is also altered in response to stress. Traditional models of stress such as immobilization, footshock, and forced swimming all decrease BDNF mRNA in the hippocampus (Smith et al., 1995; Ueyama et al., 1997). Only a few studies have examined how social stress alters BDNF in the brain. Fiore et al. (2004) found differences between dominant and subordinate aged male mice in NGF and BDNF in that dominant animals had higher levels of BDNF mRNA in the subventricular zone and hippocampus than did subordinate animals. Conversely, subordinate animals exhibited higher levels of NGF compared with dominant animals in these regions. Pizarro et al. (2004) found that BDNF mRNA in mice exposed to a 10-min social defeat was lower when compared to non-defeated animals, however, this decrease was detected in all brain regions studied (e.g., cortical and subcortical) which raises the possibility that this decrement was not region-specific. Berton et al. (2006) demonstrated that BDNF protein in the nucleus accumbens is upregulated following ten days of chronic social defeat. Finally, it was recently reported that mice that overexpress a dominant negative truncated splice variant of the BDNF receptor TrkB (TrkB.T1 mice) exhibit smaller changes in body weight and food intake than do their wildtype counterparts following repeated social defeat, yet these mice also exhibit a more consistent and long-lasting social avoidance than do the wildtype mice (Razzoli et al., 2011b). Together, these studies provide important information regarding the association of BDNF and social defeat, yet each has a potential limitation (e.g., aged animals, non-specific effects, prolonged or relatively severe defeat stress) that might limit their generalizability. It is also clear that the effect of stress on BDNF expression can differ based on the species and brain region observed as well as the stressor used. The goal of Experiment 1 was to examine the effects of exposure to an acute social interaction on BDNF mRNA in the brain of male Syrian hamsters. Experiment 1 tested the prediction that following social conflict, winning and losing animals would exhibit differences in BDNF mRNA in brain regions that are important in stress-responsivity and social behavior.

A functional role for BDNF has been demonstrated primarily in the hippocampus wherein BDNF enhances long-term potentiation (LTP; Figurov et al., 1996). In addition, BDNF-mutant mice show a deficit in LTP, an effect which can be reversed by exogenous application of BDNF (Patterson et al., 1996). Infusion of BDNF antibodies into the hippocampus prior to training results in impaired spatial learning and memory (Mu et al., 1999), and conditional TrkB knockout mice, in which the knockout of the TrkB gene is restricted to the forebrain and occurs during postnatal development, fail to successfully learn the Morris water maze (Minichiello et al., 1999).

Recently, a functional role of BDNF has also been demonstrated in regions outside of the hippocampus. For example, Rattiner et al. (2004a) showed that BDNF/TrkB signaling within the BLA is necessary for the acquisition of conditioned fear. Additionally, BDNF/TrkB signaling in the mesolimbic dopamine pathway (i.e., ventral tegmental area and nucleus accumbens) has been shown to be important for the development of social withdrawal/aversion in previously defeated mice (Berton et al., 2006).

Given that BDNF is upregulated in the BLA, a region that has been shown to be critical for fear conditioning as well as conditioned defeat (Jasnow and Huhman, 2001) the goal of Experiment 2 was to determine the functional significance of this increase. Therefore, Experiment 2 tested the hypothesis that neurotrophin signaling in the BLA is necessary for the acquisition of conditioned defeat. Unfortunately, a selective TrkB receptor antagonist does not exist so it is impossible to specifically assess, pharmacologically, the functional role of BDNF/TrkB signaling in conditioned defeat. Instead, we used K252a, a non-selective Trk receptor antagonist to evaluate the general role of neurotrophins in mediating the behavioral effects of social defeat as a first assessment of the broader hypothesis that neurotrophin receptor activation is necessary for the acquisition of conditioned defeat.

Materials and Methods

Experiment 1

Animals and Housing Conditions—Thirty adult male Syrian hamsters (*Mesocricetus auratus*) were obtained from Charles River Laboratories. Subjects weighed 120–140g at the beginning of each experiment. One week after arrival, animals were individually housed in polycarbonate cages (20 × 40 × 20cm) with wire mesh tops, corn cob bedding and cotton nesting materials in temperature-controlled (20°C ± 2°) colony rooms on a 14:10 hr light/dark cycle with lights off at 1100. Food and water were available *ad libitum*. All behavioral procedures were conducted during the first two hours of the light/dark cycle. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee, and all methods were in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Every effort was made to minimize the number of subjects used as well as to minimize any suffering by the animals.

Agonistic encounters—All animals were housed individually for two weeks and handled daily for one week prior to the start of the experiment. Animals were weight-matched and assigned to one of three groups. Sixteen animals (i.e., 8 pairs) were assigned to undergo a single, 15 min social interaction. These social conflict sessions were conducted under dim red illumination and videotaped. The total duration of the following behaviors was recorded: submissive-defensive (see below for description), aggressive (see below for description), social (attend, approach, sniff, nose-touching, and flank-marking) and non-social (locomotion, exploration, self-grooming, nesting, feeding, and sleeping). No animals were wounded during the procedure. Based on their behavior during a 15-min social defeat, which occurred in one of the animals' home cage, the subjects were characterized as either winners (Group 1) or losers (Group 2). Animals that were designated winners produced aggressive behaviors such as chasing, lunging attacks and frequent displays of upright and side offense postures. Animals that were designated losers produced submissive behaviors such as flight, tail lift, cage escape attempts, upright and side defense, and full submissive posture. No animals produced both submissive and aggressive behaviors. Animals in Group 3 served as novel cage controls and were placed in an empty animal's cage for 15-min. Animals in Group 4 were home cage controls. All animals were sacrificed 2hr following their respective treatments. This time point was selected because this is when the greatest changes in BDNF mRNA are observed following fear conditioning (Rattiner et al., 2004a)

In situ hybridization—A partial BDNF clone, containing the entire exon V coding sequence but no significant portion of the 5'-exon was subcloned from rat genomic DNA based on the NIH database sequence. This BDNF exon V clone has previously been sequenced and extensively tested (Jones et al., 2007; Rattiner et al., 2004a, b). In situ hybridization was performed as follows. Hamsters were lightly anesthetized with isoflurane gas, decapitated and their brains were removed and rapidly frozen on dry ice. Prior to sectioning, the brains were stored at –80°C. Brains were sectioned at 20µm thickness on a Leica Cryostat at –20°C onto Superfrost Plus (Fisher) slides. Sections containing anatomical areas of interest (infralimbic cortex, anterior hypothalamus, ventromedial hypothalamus, nucleus accumbens, anterior bed nucleus of the stria terminalis, posterior bed nucleus of the stria terminalis, CA1, CA3 and dentate gyrus of dorsal and ventral hippocampus, central, medial, and basolateral amygdala) were placed on 28 consecutive slides to produce four identical sets of slides for each animal. In situ hybridization was performed as previously described (Sassoon et al., 1988; Rattiner et al., 2004a, b). [³⁵S] UTP (1250Ci/ml; DuPont NEN, Boston, MA)- labeled riboprobes were prepared from linearized clones using T7 polymerase at high specific activity by only using radioactive UTP in the polymerase

reaction, with ~30% incorporation. After preparation of full-length antisense RNA strands, the RNA was base hydrolyzed to average lengths of 50–100bp and isolated using a Riboprobe spin column. Hybridizations were performed using parafilm at 52°C overnight. The slides were then stringently washed, air-dried and placed against Kodak (Rochester, NY) magnetic resonance autoradiography film for 14 days at room temperature. Optical density values of autoradiographs were obtained using MCID Basic for Windows (Imaging Research, Ontario, Canada) and calibrated using a density-step wedge (Kodak) to insure that signal intensities fell within the linear range of radioactivity. For each section, hybridization signal (optical density values) were determined bilaterally for the anatomical regions of interest (ROI) as well as an adjacent background area with little to no hybridization signal. Values for the left and right side were averaged to obtain a value for each subject. This background optical density value was then subtracted from the ROI value to produce a normalized optical density value. Normalized optical density values were calculated for two different cryostat sections for each anatomical region of interest and averaged to produce the optical density for each animal per region of interest. To reduce the possible contribution of a signal from a neighboring area, ROI delineation was limited to the innermost 90% of the total area. The anatomical level of analysis was verified for each animal using a hamster brain atlas (Morin and Wood, 2001) and sections Nissl stained with cresyl violet from the same hamster.

Statistical analysis—In Experiment 1, one-way analysis of variance (ANOVA) was used to compare optical density values for BDNF mRNA for each anatomical region of interest between winners, losers, novel cage, and home cage control animals. Because multiple comparisons were made, our alpha was corrected (Bonferroni) to control the family-wise error. For ANOVA, the alpha level was set at $p < 0.004$. Pearson correlations were used to determine an association between the duration of submissive or aggressive behavior and BDNF mRNA in regions of interest. For correlations, the alpha level was set at $p < 0.01$.

Experiment 2

Animals and Housing Conditions—Subjects were individually housed for 12–14 days prior to the start of each experiment. Older hamsters (> 6 months) that weighed 160–180 g were housed individually and used as resident aggressors during the defeat phase (see below). Younger hamsters (2 months) that weighed 100–110 g were group housed (5 hamsters per cage) and used as non-aggressive intruders during the testing phase (see below). The subjects' weights, breeder, caging, bedding, nesting materials, and food availability were as described in Experiment 1.

Stereotaxic surgery—Subjects were deeply anesthetized with sodium pentobarbital (90mg/kg) and were then bilaterally implanted with 4mm, 26-gauge guide cannulae aimed at the BLA. Lambda and bregma were leveled prior to placement of the guide cannulae. Stereotaxic coordinates were 0.4mm posterior and ± 3.9 mm lateral to bregma and 2.1mm below dura. Infusions were made with a needle that projected 4.2mm beyond the bottom of the guide cannulae. After surgery, dummy stylets were placed in the guide cannulae to help prevent clogging. All hamsters were given 10–12 days to recover from surgery before the behavioral procedure. Hamsters were handled each day following surgery by gently restraining them and removing and replacing the dummy stylet.

Social defeat and behavioral testing—The conditioned defeat model has been described elsewhere (Huhman et al., 2003). Briefly, prior to each experiment hamsters were weight-matched and randomly assigned to groups. On the training day, hamsters were transported to the behavior room. All training and testing occurred during the first 2 hr of the dark phase of the light:dark cycle. Training consisted of one, 15-min exposure to a resident

aggressor in the aggressor's home cage. Resident aggressors reliably attacked the experimental hamsters and all hamsters displayed submissive behaviors. Any hamster bitten such that it bled was removed from the study and examined by a veterinarian. The next day, a non-aggressive intruder was placed in their home cage for 5-min. The training and testing sessions were videotaped, transferred to CD-ROM, and later scored by an observer blind to the experimental conditions using behavioral scoring software (The Observer, Noldus Information Technology, Wageningen, the Netherlands). We recorded the total duration of four classes of behavior during the 5-min test: (a) submissive and defensive behavior (flee, avoidance, tail-up, upright and side defense, full submissive posture, stretch-attend, head flag, attempted escape from cage), (b) aggressive (upright and side offense, chase and attack, including bite), (c) social behavior (attend, approach, investigate, sniff, nose touching, and flank marking), and (d) non-social behavior (locomotion, exploration, self-grooming, nesting, and feeding). A reduction in conditioned defeat was indicated by a statistically significant reduction in submissive and defensive behaviors or by the return of normal territorial aggression.

Site verification—At the conclusion of the experiment, hamsters were given a lethal dose of sodium pentobarbital and infused with 300nl of India ink to verify the placement of the needle. Brains were removed and placed in 10% buffered formalin. Brains were sliced on a Leica cryostat and sections were stained with neutral red. Sections were coverslipped with DPX mountant and examined under a light microscope for evidence of ink in the BLA. Only hamsters with bilateral ink injections within 0.5mm of the BLA were included in the data analysis and determination of “hits” versus “misses” was made by two raters who were blind to experimental condition.

Drug administration—The purpose of Experiment 2 was to test the hypothesis that injection of a Trk receptor antagonist, K252a, into the BLA would reduce the acquisition of conditioned defeat. Thirty-one hamsters were matched by weight and randomly assigned to one of two conditions. Hamsters received infusions of K252a (25µg, 50µM) in 0.5µl artificial CSF (ACSF)/50% DMSO or 0.5µl vehicle control (ACSF/50%DMSO) immediately before being placed into the cage of a resident aggressor for 15-min for defeat training. Pre-training infusions of K252a at this dose into the BLA impairs fear conditioning in rats (Rattiner et al., 2004a). Infusions were administered at a rate of 0.25µl per minute with a syringe pump (Harvard apparatus PHD 2000, Natick, MA) and a Hamilton syringe connected to a 33-gauge needle via polyethylene tubing. The drug or vehicle was kept separate from the water in the tubing by a 1µl air bubble. Movement of the air bubble was monitored to assess the success of the injection procedure. Following the infusion, the needle was left in place for 2-min, then removed and replaced with the dummy stylet. On the day following defeat training, animals were tested in their own home cage against a non-aggressive intruder for 5-min.

Statistical analysis—The total durations of submissive/defensive, social and non-social behavior emitted during the testing session were analyzed using an independent samples t-test. No comparisons of aggression were planned as defeated subjects generally exhibit little to no aggression during testing. The criterion for significance was $p < 0.05$.

Results

Experiment 1

One pair of animals did not exhibit any agonistic behavior and did not establish a strong winner/loser relationship. Thus, a total of 28 animals were used in the analysis: winners (n=8), losers (n=8), novel cage control (n=6), and home cage control (n=6). Figure 1 shows

the group means for the optical densities of BDNF mRNA in the BLA, MeA, DHPC DG and VHPC CA1. There was a significant difference in BDNF mRNA in the BLA among winners, losers, novel cage and home cage controls 2 hours after the social interaction ($F_{(3, 27)} = 34.38$, $P < 0.001$, Figure 2). Post-hoc analysis revealed that losers had more BDNF mRNA in the BLA than did winners, novel cage and home cage controls. In addition, winners had more BDNF mRNA in the BLA than did novel cage and home cage controls (Figure 1).

A significant difference in BDNF mRNA in the MeA among the groups was also detected ($F_{(3, 27)} = 30.85$, $P < 0.001$, Figure 2). Post-hoc analysis showed that losers had higher BDNF mRNA levels in the MeA than did winners, novel and home cage controls. Winning animals had higher BDNF mRNA levels than did novel and home cage controls. Finally, novel cage controls had higher BDNF mRNA levels than did home cage controls (Figure 1).

There was also a significant difference among the groups in BDNF mRNA in the dentate gyrus of the dorsal hippocampus (DHPC DG, $F_{(3, 27)} = 8.47$, $P = 0.001$, Figure 2). Post-hoc testing revealed that winners had more BDNF mRNA in DHPC DG than did losers, novel, and home cage controls (Figure 1).

No significant differences among the groups were detected in BDNF mRNA in the following regions: infralimbic cortex ($F_{(3, 27)} = 0.50$, $P > 0.05$), anterior hypothalamus ($F_{(3, 27)} = 0.177$, $P > 0.05$), ventromedial hypothalamus ($F_{(3, 27)} = 0.11$, $P > 0.05$), nucleus accumbens ($F_{(3, 27)} = 0.85$, $P > 0.05$), anterior bed nucleus of the stria terminalis ($F_{(3, 27)} = 0.96$, $P > 0.05$), posterior bed nucleus of the stria terminalis ($F_{(3, 27)} = 0.75$, $P > 0.05$), DHPC CA1 ($F_{(3, 27)} = 0.42$, $P > 0.05$), DHPC CA3 ($F_{(3, 27)} = 0.61$, $P > 0.05$), VHPC CA1 ($F_{(3, 27)} = 3.06$, $P = 0.047$), VHPC CA3 ($F_{(3, 27)} = 0.11$, $P > 0.05$), VHPC DG ($F_{(3, 27)} = 0.62$, $P > 0.05$), and central amygdala ($F_{(3, 27)} = 0.28$, $P > 0.05$).

A significant, positive correlation was found between the duration of submissive behavior and BDNF mRNA in the MeA ($r(12) = 0.721$, $P = 0.004$). A significant, negative correlation was detected between duration of aggressive behavior and BDNF in the BLA ($r(11) = -0.742$, $P = 0.004$) as well as the duration of non-social behavior and BDNF mRNA in the MeA ($r(12) = -0.691$, $P = 0.006$).

Experiment 2: Acquisition of conditioned defeat

Effects of infusion of K252a into the BLA on the acquisition of conditioned defeat—A total of 20 animals with bilateral cannula placements in the BLA were used in the statistical analysis: vehicle ($n = 9$), K252a ($n = 11$). A histological reconstruction of the injection sites is shown in Figure 3. The duration of submissive/defensive behavior during testing was significantly reduced in animals that received infusion of K252a immediately before defeat training when compared to animals that received vehicle, $t(18) = 2.789$, $p < 0.05$ (Figure 4). There were no significant differences between animals that received K252a or vehicle in social and non-social behavior. Eleven animals were excluded before the statistical analysis. Two animals lost their cannula during the recovery period and could not be used in the study. Seven animals had injections that were not localized to the BLA. Two of these animals had bilateral placements in the central amygdala (capsular) while one had unilateral placements in the central amygdala with BLA placement on the other side. Two animals had bilateral placements in the ventral endopiriform nucleus and one had unilateral placement in the ventral endopiriform nucleus on one side with BLA placement on the other side. One animal had unilateral placement in the posterior basomedial amygdalar nucleus on one side with BLA placement on the other side. Two animals did not receive injections as a result of obstructed cannulae. These animals were examined as “miss control groups” to assess anatomical specificity of the behavioral results. Of the animals that were considered

bilateral anatomical “misses”, three received K252a infusion and one received vehicle infusion. The animals that received K252a showed levels of submissive and defensive ($M = 55.04$, $SEM = 11.34$) behaviors during the testing session that were comparable to that of vehicle controls. Of those animals considered unilateral anatomical “misses”, three received K252a infusions and one received vehicle infusions. These animals also showed levels of submissive and defensive behaviors comparable to vehicle controls ($M = 65.03$, $SEM = 8.1$).

Because K252a infused prior to the training session reduced submissive and defensive behaviors during testing, one might argue that such a reduction could be due to the fact that the training session for animals that received vehicle was different from that experienced by animals that received K252a (i.e., the behavior of the resident aggressor was different depending on the treatment received by its opponent). To address this issue, the behavior of the resident aggressor was scored during each training session to ensure that both groups of subjects experienced comparable levels of aggression. Resident aggressors exhibited an average of 633 ($SEM = 39$) sec of aggression towards animals receiving K252a and 606 ($SEM = 74$) sec of aggression towards animals receiving vehicle. Thus, all subjects received high levels of aggression and no significant difference in the behavior of the aggressors towards the two groups was noted during defeat training: aggressive [$t(18) = 6.26$, $P > 0.05$], social [$t(18) = 7.33$, $P > 0.05$] and non-social [$t(18) = 6.43$, $P > 0.05$]. All experimental animals, regardless of treatment, responded to the initial defeat training with high levels of submissive/defensive behaviors.

Discussion

Experiment 1: Agonistic behavior alters BDNF mRNA in the BLA, MeA, and DHPC DG

The results of Experiment 1 indicate that exposure to an agonistic encounter is capable of producing specific changes in BDNF mRNA in a variety of brain regions. Losing, winning, novel and home cage control animals displayed differences in BDNF mRNA in the BLA, MeA, and DHPC DG but not in the infralimbic cortex, anterior hypothalamus, ventromedial hypothalamus, ventral hippocampus, nucleus accumbens, bed nucleus of the stria terminalis and central amygdala. In some cases, the duration of a particular behavior (i.e., submissive, aggressive, non-social) correlated either positively or negatively with the amount of BDNF mRNA in particular brain regions.

Our finding that exposure to agonistic interactions increases BDNF mRNA in the BLA is not necessarily consistent with previous studies which have shown that a variety of non-social stressors (e.g., immobilization or restraint) and exposure to social defeat *decrease* BDNF mRNA (Smith et al., 1995; Xu et al., 2004; Pizarro et al., 2004). There are several important methodological differences that might explain this inconsistency. First of all, it is possible that the BDNF mRNA response to a social stressor in which behavioral plasticity occurs (e.g., conditioned defeat) might be very different from the response to a non-social stressor. Pizarro et al. (2004), however, showed that mice exposed to social defeat exhibited decreased BDNF mRNA in several cortical and subcortical regions, including the BLA, 24 hours following defeat. Methodological differences between the social defeat models employed, particularly the 24hr (Piazza) versus the 2hr (current study) sampling period, and this difference in timing might explain the opposite response of BDNF mRNA. A significant literature now suggests that BDNF is rapidly upregulated during the consolidation period following various forms of learning including emotional fear learning (Jones et al., 2007, Rattiner, et al. 2004a, b). Thus, it is quite possible that BDNF mRNA might increase immediately following a stressor as part of the synaptic plasticity mediating memory consolidation while it is reduced 24 hr later via a different mechanism mediating the long-term effects of stress. Furthermore, hamsters in the current study underwent an acute social defeat by a single opponent while mice in the Pizarro study were exposed to three defeat

sessions by three different aggressors. It is possible that exposure to this more severe form of social stress results in decreased BDNF levels, while less intense forms, such as an acute social defeat in hamsters, result in an increase in BDNF. It is also important to note that we included a novel cage control group in which hamsters were exposed to an empty aggressor's cage for 15 minutes. The inclusion of this group in addition to a home cage control allowed us to demonstrate that the changes we observed in both winning and losing hamsters were specific to social interaction and fight outcome and were not due simply to exposure to a novel environment with chemosensory cues from a conspecific.

Data from our laboratory suggest that at least some of the plasticity underlying conditioned defeat occurs in the BLA. For example, over-expression of CREB in the BLA enhances the memory of social defeat (Jasnow et al., 2005), while pretraining BLA infusions of ifenprodil (an NMDA NR2B subunit antagonist; Day et al., 2011) or anisomycin (a protein synthesis inhibitor; Markham and Huhman, 2008) both reduce the behavioral effects of social defeat. The results of this experiment are consistent with the hypothesis that plasticity in the BLA mediates, at least in part, the behavioral changes observed following defeat and suggest that BDNF may be an important molecular mediator of these changes. The finding that losing animals had higher levels of BDNF mRNA in the MeA than did winning animals, novel and home cage controls and that winners also had higher BDNF mRNA than controls is also interesting given the role of the MeA in processing chemosensory information. This increase suggests that the MeA may be an important site of plasticity following social interactions not only in losers and also in winners. We have previously demonstrated, however, that pre-training infusion of anisomycin into the MeA does not reduce the behavioral effects of social defeat (Markham and Huhman, 2008), suggesting that protein synthesis in the MeA is not necessary for defeat-induced behavioral plasticity. Hamsters rely heavily upon olfactory functioning to avoid predation, to identify mating partners and in some cases to recognize a conspecific (Petrulis et al., 2004). Interestingly, novel cage control animals also exhibited higher levels of BDNF mRNA in the MeA than did home cage controls, suggesting that exposure to a novel social environment, alone, induces changes in BDNF. In addition, BDNF mRNA levels were higher in all animals that engaged in a fight (i.e., winners and losers) than they were in both control groups. It may be the case that all situations involving exposure to novel odor stimuli induces some degree of plasticity in the MeA to effectively encode chemosensory information relevant to potential social interaction but that this plasticity is not critical for future conflict-induced behavioral changes, per se. Instead, the degree of BDNF production may be directly related to the degree of emotional arousal engendered by the particular situation; thus, BDNF mRNA is increased in the mildly stressful novel cage, but is increased more in the winning hamsters and even more in hamsters that fight and lose.

Our data also revealed differences in BDNF mRNA in DHPC DG in winners, novel and home cage controls. Most studies that examine changes in BDNF mRNA levels following stress, including social stress, report decreases in BDNF mRNA in the hippocampus (Smith et al., 1995, Nibuya et al., 1995, Pizarro et al., 2004). Again, most of these studies involve chronic stress or examination of BDNF following a prolonged post-stress interval, in contrast to our current study wherein we examined BDNF during the consolidation period following an acute emotional learning event. We found that winning animals had significantly higher BDNF mRNA levels in DHPC DG than did losers, novel and home cage controls. This finding suggests that the behaviors associated with aggression and winning a fight may also involve plastic mechanisms. In other words, the finding that DHPC DG BDNF levels were greater in winners and BLA BDNF levels were greater in losers may be consistent with the notion that following winning it is important to encode spatial representations involved in the instantiation and defense of a potential new territory, whereas losing may primarily activate fear and flight circuitry.

Finally, we applied several correlation analyses to detect a relationship between the duration of a particular behavior class and the amount of BDNF mRNA signal in specific brain regions. We found that high levels of aggressive behaviors correlated with lower levels of BDNF mRNA in the MeA and BLA. While no significant relationship was detected between duration of submissive behavior and BDNF mRNA levels in the BLA, it is possible that the experience of social defeat upregulated BDNF to support the behavioral changes associated with losing. Interestingly, a positive correlation was detected between duration of submissive behavior and BDNF mRNA in the MeA. This finding again suggests that there is important plasticity that may occur in the MeA to support defeat-induced behavioral changes. A negative correlation was detected between duration of submissive behavior and BDNF mRNA in DHPC DG, suggesting that learning about context may not be an important factor or conditioned stimulus that is instantiated in the formation of conditioned defeat. This is consistent with the fact that conditioned defeat readily generalizes to contexts that are very different from that in which the initial defeat occurred. Indeed, in most of our studies the initial defeat occurs in the home cage of a larger, more aggressive “trainer”, whereas testing for conditioned defeat subsequently occurs in the home cage of the subject.

Overall, these findings may be most consistent with a model in which: 1) BDNF is actively regulated at a transcriptional level during the memory consolidation period in brain regions involved in the emotional, and perhaps contextual, learning that occurs following social conflict, 2) emotional learning induces the largest increases in BDNF in amygdala regions (BLA and MeA) in animals when social conflict results in losing and the emergence of submissive behaviors, and 3) social conflict-induced learning leads to the largest increases in BDNF in hippocampal regions (DHPC DG) when the conflict results in winning and the continuation or strengthening of territorial aggressive behaviors. Interestingly, it is also apparent that winning is associated with significant, albeit smaller, increases in BDNF mRNA in the amygdala (BLA and MeA), suggesting that the amygdala can also instantiate behavioral changes following the experience of winning.

Experiment 2: Infusion of K252a reduces the acquisition of conditioned defeat

The results of Experiment 2 indicate that neurotrophin activity in the BLA is important for the acquisition of conditioned defeat. The finding that blockade of Trk receptors during the initial social defeat session reduces the display of submissive and defensive behaviors 24-hours later during the testing session suggests that neurotrophic activity in the BLA is important for learning or encoding information about losing a fight. An alternative explanation is that the treatment altered the levels of agonistic behavior during the training session such that animals receiving vehicle were defeated differently than those that received K252a. In other words, it is possible that resident aggressors behaved differently towards drug animals. We maintain that this is not the case, however, because all animals received similar defeats regardless of treatment group (see results for means of aggressive behavior elicited by RAs towards drug and vehicle groups).

This study is among the first to demonstrate a functional role for neurotrophic factors in the behavioral adaptations that occur following social defeat. Although K252a is a non-selective neurotrophin receptor antagonist, it is very possible that BDNF in the BLA mediates at least some of the behavioral responses to social stress. Certainly, the finding that TrkB.T1 mice exhibit changes in the behavioral response to social defeat is consistent with this possibility (Razolli et al., 2011). It is also possible that other neurotrophins, such as nerve growth factor, play a role in these behavioral changes.

Conclusions

Exposure to social and non-social stressors has been shown to alter both the brain and behavior. The study of how biologically relevant stressors, such as social defeat, effect the brain and behavior is important for understanding the pathology that underlies fear and anxiety disorders such as post-traumatic stress disorder (PTSD). Here, we show that agonistic encounters in hamsters can alter BDNF mRNA in specific brain regions that are important in stress-responsivity, fear, social behavior, and learning. Furthermore, we demonstrate that neurotrophic activity in the BLA is important for the acquisition of conditioned defeat.

In conclusion, studies of BDNF show promise in elucidating the mechanisms by which social stress produces alterations in the brain and subsequent behavior. Ongoing studies in our laboratory are focusing on the plastic mechanisms that occur within the neural circuit underlying conditioned defeat.

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Figure 1. Mean ($\pm$ standard error of the mean) of BDNF mRNA in the BLA, MeA, and DHPC DG in losers, winners, and novel and home cage controls. Unshared letters indicate a significant difference among groups ($P < 0.004$).

Figure 2. BDNF mRNA in the BLA, MeA, and DHPC DG 2hr after exposure to an agonistic encounter at low and high power magnification.

Figure 3. Histological reconstructions of injection sites of animals receiving infusions into the BLA in Experiment 2. Black dots represent the site of injection in one or more animals. Black triangles represent anatomical misses. Drawings are adapted from Morin and Wood (2001).

Figure 4.

Acquisition of conditioned defeat. Total duration (mean $\pm$ S.E.M.) of submissive/defensive, aggressive, social, and nonsocial behavior displayed by defeated hamsters during a 5-min test with a non-aggressive intruder. Animals received bilateral infusions of vehicle or K252a into the BLA immediately before being defeated by a resident aggressor for 15 min on the previous day. Asterisk indicates a significant difference from vehicle ($p < 0.05$).