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INTERMITTENT SOCIAL DEFEAT STRESS ENHANCES MESOCORTICOLIMBIC ΔFOSB/BDNF CO-EXPRESSION AND PERSISTENTLY ACTIVATES CORTICOTEGMENTAL NEURONS: IMPLICATION FOR VULNERABILITY TO PSYCHOSTIMULANTS

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Abstract—Intermittent social defeat stress exposure augments behavioral response to psychostimulants in a process termed cross-sensitization. Brain-derived neurotrophic factor (BDNF) mediates synaptic plasticity and cellular responses to stress and drugs of abuse. We previously showed that repeated social defeat stress persistently alters BDNF and activates ΔFosB expression in mesocorticolimbic regions. Here, we hypothesized that social defeat stress would increase ΔFosB expression in BDNF-containing mesocorticolimbic neurons at a time when cross-sensitization is evident. Because the ventral tegmental area (VTA) is critical for cross-sensitization, we similarly hypothesized that repeated social defeat stress would induce ΔFosB in neurons of mesocorticolimbic terminal regions that innervate the VTA. We induced social defeat stress in rats by short confrontations with an aggressive resident rat every third day for 10 days. Control rats were handled according to the same schedule. Defeated rats exhibited sensitized locomotor response to amphetamine (1.0 mg/kg, i.p.) 10 days after termination of stress exposure. Separate rats, which underwent stress procedures without amphetamine challenge, were used for histological assessments. Rats received intra-VTA infusion of the retrograde tracer, Fluorogold (FG), and brain tissue was collected 10 days after stress or handling for immunohistochemistry. Stress exposure increased BDNF immunoreactivity in anterior cingulate, prelimbic and infralimbic regions of the prefrontal cortex (PFC), medial amygdala (AMY), nucleus accumbens (NAc) and VTA; ΔFosB labeling in anterior cingulate cortex (ACG) and nucleus accumbens; and ΔFosB/BDNF co-expression in prelimbic cortex (PL), nucleus accumbens and medial amygdala. Infralimbic ΔFosB-labeling was enhanced by stress in neurons innervating the VTA. Increased ΔFosB/BDNF co-expression and persistent functional activation of corticolimbic neurons after stress may contribute to mechanisms underlying cross-sensitization to psychostimulants.

Key words: social defeat stress, vulnerability, amphetamine, cross-sensitization, prefrontal cortex, amygdala.

INTRODUCTION

Pre-clinical and clinical data point to stress exposure as a risk factor for addictive behavior (Sinha, 2007). Social stress resulting from defeat after aggressive confrontations with conspecific counterparts is a powerful stressor for both humans and animals (Koolhaas et al., 1999; Björkqvist, 2001). In rodent models, social defeat stress induces profound and long-lasting alterations of function in mesocorticolimbic circuits accompanied by persisting enhancement of drug-related behaviors. This includes augmented behavioral responses to low doses of psychostimulants, a process termed cross-sensitization, and enhanced psychostimulant self-administration (Covington and Miczek, 2001; Nikulina et al., 2004; Covington et al., 2005).

Behavioral sensitization is a consequence of drug-induced neuroadaptive changes and is thought to underlie certain aspects of drug addiction, such as craving and relapse (Robinson and Berridge, 2001). A neural circuit involving dopaminergic and glutamatergic interconnections between the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) is essential for the induction and expression of behavioral sensitization (Vanderschuren and Kalivas, 2000). Brain-derived neurotrophic factor (BDNF) is a neurotrophin important for synaptic plasticity (Kang and Schuman, 1995; Horch et al., 1999) that is expressed within these regions (Seroogy et al., 1994; Conner et al., 1997) and may represent a critical molecular stimulus for persisting psychomotor cross-sensitization.

Social defeat stress induces activation of the mesocorticolimbic dopamine system (Tidey and Miczek,

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Abbreviations: ACG, anterior cingulate cortex; AMY, amygdala; BDNF, brain-derived neurotrophic factor; FG, fluorogold; IL, infralimbic cortex; NAc, nucleus accumbens; PFC, prefrontal cortex; PL, prelimbic cortex; VTA, ventral tegmental area.

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1996), and stimulation of dopamine synthesis promotes the expression of BDNF (Okazawa et al., 1992). Recently we observed that repeated social defeat stress increases short-term BDNF expression in prefrontal cortical regions and delayed, prolonged BDNF expression in medial AMY and VTA (Fanous et al., 2010). Similarly, repeated exposure to psychostimulants both produces behavioral sensitization and increases BDNF in the PFC, NAc, and AMY (Meredith et al., 2002; Le Foll et al., 2005; Fumagalli et al., 2007; Fanous et al., 2011). These lines of evidence suggest that stress-induced alteration of BDNF signaling in these brain regions could regulate the function of this reward circuit (Ghitza et al., 2010).

Additionally, inducible transcription factors of the Fos family are involved in neuroadaptations resulting from stress or psychostimulant administration (Hope et al., 1994; Vanderschuren et al., 2002; Perrotti et al., 2004; Hope et al., 2006; Perrotti et al., 2008). ΔFosB, a stable protein of the Fos family induced by chronic drug treatments, has been proposed as an important mediator of long-term plasticity in the brain (Nestler et al., 1999; McClung et al., 2004; Nestler, 2008). We previously observed that repeated social defeat stress increases ΔFosB expression in mesocorticolimbic terminal regions such as the PFC, NAc, and AMY, which persists up to 14 days after stress termination (Nikulina et al., 2008). Because enhanced mesocorticolimbic BDNF and ΔFosB represent lasting molecular consequences of both repeated social defeat stress and chronic drug treatments, expression of both together may contribute to cross-sensitization. However, whether ΔFosB and BDNF are co-expressed in mesocorticolimbic neurons during cross-sensitization is unknown.

Our present aim was to examine anatomical substrates for prolonged molecular consequences of social defeat stress. We hypothesized that repeated activation of mesocorticolimbic neurons by social defeat stress would increase ΔFosB and BDNF in an overlapping population of neurons. Thus, ΔFosB and BDNF co-expression was examined in mesocorticolimbic regions 10 days after exposure to intermittent social defeat stress at a time when behavioral cross-sensitization to psychostimulants is known to be present. Additionally, we hypothesized that stress exposure would increase ΔFosB in prefrontal cortical neurons innervating the VTA, which is implicated in the development of behavioral sensitization (Kalivas and Weber, 1988; Perugini and Vezina, 1994) and is reciprocally connected to PFC (Geisler and Zahm, 2005). To investigate this, we infused the retrograde tracer Fluorogold (FG) into the VTA and measured ΔFosB/FG co-labeling in mesocorticolimbic terminal regions 10 days after repeated social defeat stress exposure.

EXPERIMENTAL PROCEDURES

Subjects

Twenty-nine male Sprague–Dawley rats (Charles River Laboratories, Hollister, CA, USA) were acclimated to laboratory conditions for one week prior to the start of experimentation. Rats weighed 270–300 g at the beginning of experimental manipulations, and were singly housed in standard plastic cages (55 × 31 × 21 cm) prior to behavioral procedures and during recovery from surgery. Rats were maintained under a reverse 12-h light–dark cycle (lights off at 0900 h) with free access to food (Purina Rodent Chow) and water. Male hooded Long-Evans rats (weighing 550–700 g), termed ''residents,'' were continuously pair-housed with an individual female in large plastic cages (37 × 50 × 20 cm), and were used to induce social defeat stress in experimental Sprague–Dawley male rats. All females underwent tubal ligation prior to pair-housing with males to maintain cycling and prevent pregnancy. Residents were screened repeatedly for reliable performance of aggressive behavior toward an intruder rat. All experimental procedures were approved by the University of Arizona and Arizona State University Institutional Animal Care and Use Committees, and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2003). In addition, all efforts were made to minimize animal suffering and the number of subjects.

Experimental design

General procedure. After adaptation to laboratory conditions, rats were randomly assigned to either the ''social defeat stress'' experimental group or to a non-stressed handled control group. Experimental rats were exposed to intermittent social defeat stress every third day for 10 days (4 defeats total), and control rats were handled at the same times, but not exposed to resident rats. Two experiments were conducted in parallel. One set of rats (n = 17) was subjected to social defeat stress to induce behavioral cross-sensitization or to control handling, and 10 days later was challenged with amphetamine. We chose this time-point because we and others have demonstrated both behavioral cross-sensitization and alterations in Fos and BDNF response in various mesocorticolimbic regions 10 days after repeated social defeat (Nikulina et al., 2004; Miczek et al., 2008; Miczek et al., 2011). In order to assess persistent protein changes resulting from stress without the confound of an acute amphetamine challenge, another set of rats (n = 12) received injections of FG into the VTA one week before the same social defeat stress or handling procedure, and were euthanized 10 days after stress termination, a time point corresponding to the expression of cross-sensitization. Brain tissue was collected in the absence of amphetamine challenge in order to assess long-term effects of repeated social defeat stress on BDNF and ΔFosB expression at a time when cross-sensitization to amphetamine would normally be expressed.

Surgeries. Rats used for histological assessments received unilateral FG injections into VTA at the following stereotaxic coordinates: AP = −5.1, DV = −8.8, ML = −0.6 (Paxinos and Watson, 2005). FG produces reliable and long-lasting retrograde labeling from discrete injection sites without disrupting behavior or subsequent histochemical procedures (Cheung and Hammer, 1995). Briefly, FG (4% in 0.1 M sodium cacodylate buffer; Fluorochrome, LLC, Denver, CO) was infused into rats anesthetized with isofluorane by stereotaxic iontophoresis using a 20–30 μm tip diameter micropipette and 6 μamp, 7 s alternating current for 10 min. Withdrawal of the micropipette was accompanied by application of −1 μamp current to avoid FG diffusion along the pipette track. Wounds were closed with bone wax and surgical staples. Behavioral manipulation with social defeat stress or handling began after one week of recovery.

Social defeat stress. Experimental rats were defeated as described previously (Tornatzky and Miczek, 1995; Nikulina et al., 2004). After removing the female from the resident's cage 30 min prior to social stress exposure, the experimental intruder rat was placed into the home cage of a resident male rat. For