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Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression

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Abstract

Components of the brain's dopaminergic system, such as dopamine receptors, undergo final maturation in adolescence. Exposure to social stress during human adolescence contributes to substance abuse behaviors. We utilized a rat model of adolescent social stress to investigate the neural mechanisms underlying this correlation. Rats exposed to repeated social defeat in adolescence (P35–P39) exhibited increased conditioned place preference (CPP) for amphetamine (1 mg/kg) in adulthood (P70). In contrast, rats experiencing foot-shock during the same developmental period exhibited amphetamine CPP levels similar to non-stressed controls. Our previous experiments suggested adolescent defeat alters dopamine activity in the mesocorticolimbic system. Furthermore, dopamine receptors have been implicated in the expression of amphetamine CPP. Therefore, we hypothesized that alteration to dopamine receptor expression in the mesocorticolimbic system may be associated with to heightened amphetamine CPP of adult rats exposed to adolescence defeat. We measured D1 and D2 dopamine receptor protein content in the medial prefrontal cortex, nucleus accumbens (NAc) and dorsal striatum following either adolescent social defeat or foot-shock stress and then adult amphetamine CPP. In controls, amphetamine CPP training reduced D2 receptor protein content in the NAc core. However, this down-regulation of NAc core D2 receptors was blocked by exposure to social defeat but not foot-shock stress in adolescence. These results suggest social defeat stress in adolescence alters the manner in which later amphetamine exposure down-regulates D2 receptors. Furthermore, persistent alterations to adult D2 receptor expression and amphetamine responses may depend on the type of stress experienced in adolescence.

Keywords

social stress; psychostimulant; adolescence; D2 dopamine receptor; D1 dopamine receptor; contextual cues

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1.0 Introduction

Approximately 10 percent of all adolescents are repeatedly victimized by peers (Nansel *et al.*, 2001), and this can initiate substance abuse behaviors (Nelson et al., 1995, Hoffmann et al., 2000, Tharp-Taylor et al., 2009). Exposing laboratory rats to repeated social defeat is used to model human bullying behavior (Bjorkqvist, 2001), and adult social defeat causes subsequent cross-sensitization to psychostimulant reward-related behaviors (Haney et al., 1995, Miczek et al., 2004, Covington et al., 2008). However, the effects of social defeat in adolescence on drug-related behaviors have received relatively little attention.

Rats exposed to social defeat in adolescence show increased locomotion in novel environments and decreased basal and amphetamine-induced dopamine (DA) in the medial prefrontal cortex (mPFC) as adults, accompanied by an enhanced DA response to amphetamine in the nucleus accumbens (NAc) core (Watt et al., 2009, Burke et al., 2010). Rats that exhibit heightened locomotion in a novel environment (termed high responders) also show blunted basal DA activity in the mPFC and elevated NAc DA responses to cocaine (Hooks et al., 1991b, Piazza et al., 1991, Hooks et al., 1992a). High responder rodents also exhibit greater psychostimulant sensitization (Hooks et al., 1991a), self-administration (Piazza et al., 1989) and conditioned place preference (CPP) (Orsini et al., 2004). Given a similar novelty and neurochemical profile to high responders, rats exposed to social defeat in adolescence may also show heightened drug behaviors in adulthood. However, the effects of adolescent social defeat stress on later amphetamine CPP or sensitization have not been determined.

Within the mesocorticoaccumbal and striatal systems, both D1 and D2 DA receptors undergo essential maturation or pruning during adolescence (Andersen et al., 2000, Tarazi and Baldessarini, 2000). Furthermore, both D1 and D2 receptors have been a major mechanism of focus in addiction research (Anderson and Pierce, 2005). For example, D2 receptors in the striatum and NAc have been inversely correlated with addiction vulnerability and in humans and rats (Hooks et al., 1994, Volkow et al., 2004). Also, increased D1 receptor protein levels in the NAc were observed following repeated twice daily cocaine injections (Unterwald et al., 2001), suggesting that repeated psychostimulant exposure may enhance synaptic responsiveness to subsequent dopamine release. Activation of D1 and D2 receptors is also important for stress cross-sensitization to amphetamine CPP (Capriles and Cancela, 1999). Therefore, it is possible that potential alterations in amphetamine CPP as a result of adolescent social defeat may be reflected by changes to dopamine receptor expression in the mesocorticoaccumbal system.

In this study, we investigated the effects of adolescent social defeat on adult amphetamine CPP, and whether this stressor combined with amphetamine conditioning also altered adult DA receptor expression. To elucidate the specificity of social stress exposure during adolescent development in causing these changes, we also investigated the effect of adolescent foot-shock stress on the same adult measures.

2.0 Experimental Procedures

2.1 Animals

Male Sprague-Dawley rats (Animal Resource Center, Univ. South Dakota, Vermillion, SD) used as experimental animals were pair-housed under a reverse light cycle (lights off from 10:00–22:00 hrs) on postnatal day (P) 21. Resident adult male Sprague-Dawley rats (300–400g) for social defeat experiments were housed singly prior to aggressive screening. Food and water was available *ad libitum*. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of South Dakota, and were

carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All testing procedures were conducted between 11:00 and 18:00 under red lighting.

2.2 Social Defeat

Social defeat procedures used here followed previous methods which demonstrated that social defeat initiated at P35 resulted in alterations to dopamine systems and amphetamine-induced dopamine and corticosterone responses (Watt et al., 2009, Burke et al., 2010). Sexually receptive females were used to induce territorial aggression from the residents prior to the defeat episodes. For social defeat procedures, an adolescent male rat (P35) was introduced into the resident's home cage in the absence of the female rat. The adolescent rat was considered socially defeated when it exhibited three submissive postures (defined by Blanchard et al., 1993, Miczek and De Boer, 2005) in response to resident attacks. The average time ($\pm$ SEM) to reach this social defeat criterion was 2.71 $\pm$ 0.13 min. Following this, the resident and the adolescent subject were separated for 35 min by a mesh barrier, which prevented further physical attacks but still allowed for further visual, auditory and olfactory intimidation from the resident (Watt et al., 2009, Burke et al., 2010). Adolescents experienced social defeat each day for 5 consecutive days ($N = 30$) and were exposed to a different resident male each day to control for variance in defeat intensity. Age-matched controls ($N = 30$) were placed in empty novel cages for the duration of each defeat episode to control for handling and novel environment stress (Watt et al., 2009, Burke et al., 2010). After repeated defeat or novel cage exposure, all rats were allowed to mature undisturbed into early adulthood (P56) when acclimation to the CPP apparatus began.

2.3 Foot-shock

Foot-shock stress was administered daily to a separate group of rats ($N = 22$) during adolescence (P35) for 5 consecutive days. Foot-shock chambers (30 cm $\times$ 30 cm; Noldus Information Technology, Leesburg, VA) contained a speaker to deliver tone and an overhead camera that relayed information to a computer and video observation software (Ethovision 3.1, Noldus Information Technology). The floor consisted of bars (0.48 cm diameter 1.59 cm apart) that delivered experimenter-controlled foot-shock. Each foot-shock chamber was enclosed in a dark sound-attenuating chamber (Med Associates, Inc, St. Albans, VT). Rats were acclimated to the chamber for 5 min each day, then presented with 5 s tone (56 dB) terminating with a 0.5 s foot-shock (0.5 mA), which was repeated every minute for 10 min (Lukkes et al., 2009). Controls ($N = 22$) were placed in the same chamber and exposed to the tone in the absence of shock.

2.4 Conditioned Place Preference (CPP)

Amphetamine CPP was chosen since this is a well-established method for assessing an animal's motivation to prefer a context paired with drug experience (Tzschentke, 1998, Bardo and Bevins, 2000, Tzschentke, 2007). Dopamine activity in the NAc and striatum has been implicated in preference for cues paired with amphetamine in rats and humans (Boileau et al., 2007, Schiffer et al., 2009). Furthermore, blocking D1 or D2 receptors prevents stress-induced enhancement of amphetamine CPP, suggesting D1 and D2 receptor involvement in stress cross-sensitization to amphetamine CPP (Capriles and Cancela, 1999). The CPP apparatus (Med Associates, Inc, St Albans, CT) consisted of three chambers (20.96 cm W $\times$ 20.93 cm H $\times$ 27.94 cm L), one white with a steel mesh floor (1.27 $\times$ 1.27 cm holes) and the other black with a bar floor (0.48 cm diameter 1.59 cm apart). The chambers were separated by a small center chamber (20.96 cm W $\times$ 20.93 cm H $\times$ 12.07 cm L) with grey plastic walls and floor. A ceiling light remained on in the center chamber to discourage time spent in this area. Acclimation to the CPP apparatus began by placing the rat (at P56) into the center compartment and allowing it to explore the entire apparatus freely for 30 min on each of 3

days. Photobeams within the apparatus recorded time spent and movement within each chamber. Time spent in each chamber during acclimation was examined to identify any rats that displayed a pre-preference (more than 50% time spent) for a particular chamber. To allow an unbiased design, 13 of the total 104 rats were removed from the study prior to any amphetamine or saline pairings since they exhibited a pre-preference for one of the chambers (Tzschentke, 1998, 2007).

Rats were randomly chosen to receive amphetamine (1 mg/kg, ip.) paired with one chamber and saline in the other, or to receive saline injections paired with both chambers. An equal number of pre-stressed and control rats were randomly assigned to each group, creating a counterbalanced group assignment procedure (Tzschentke, 2007). The concentration of amphetamine used (1 mg/kg) has been shown to elicit greater CPP in high but not low novelty seeking rats (Kebaur & Bardo, 1999). The social defeat experiment comprised 15 rats per treatment group, and the foot-shock experiment comprised 9–10 rats per group. On conditioning days each rat was placed into the appropriate chamber for 30 min immediately following saline or amphetamine injection. Five pairings of amphetamine (saline for drug control groups) and 5 pairings of saline with the respective chamber on alternate days totaled 10 days of conditioning. The following day (day 11), rats were placed in the center of the apparatus in the absence of an injection, and allowed to explore the entire apparatus freely with the time spent in each compartment recorded.

2.5 Western Blots

One day following CPP testing, rats were decapitated with brains rapidly removed and frozen at -80°C . Brains were cut in 300 μm sections at -10°C using a Leica-Jung cryostat (North Central Instruments MN), thaw-mounted on glass slides, and stored at -80°C . The mPFC, NAc shell, NAc core, and striatum were identified using Paxinos and Watson's (1998) rat brain atlas. All dissections were performed bilaterally with a 430 μm (NAc) or 580 μm (striatum and mPFC) inside diameter cannula on a freezing stage (Physitemp, Clifton, NJ), and samples were expelled into 40 μl of HEPES buffer. Tissue was homogenized briefly with a sonic dismembrator (Model 100, Fisher Scientific, Pittsburgh, PA). Protein content within 5 μl sample duplicates was determined using a Bradford Kit (BioRad Laboratories, Hercules, CA) and an automated microplate reader (Bio-Tek Instruments, Winooski, VT). Samples (40 μg protein for striatum and NAc, 70 μg protein for mPFC) were subjected to western blotting by SDS–polyacrylamide gel (10% Acrylamide/Bis) electrophoresis (BioRad Laboratories) and subsequent transfer to PVDF membranes (BioRad Laboratories). Membranes were blocked in 5% non-fat dry milk (NFDm) for 1 hour prior to being incubated with an antibody recognizing amino acids 9–21 of the D1 receptor (rabbit anti-D1; 1:1000; ab40653, Abcam, Cambridge, MA) and a 28 amino acid sequence within the third intracellular loop of D2 receptor (rabbit anti-D2 1:300; AB5084P, Millipore, Billerica, MA) at 4°C for 18 hrs. The D1 receptor antibody labeled one band only, at the 49 kDa weight as others have found with different D1 receptor antibodies (Naha et al., 2009, Sun et al., 2009). The D2 receptor antibody produced one band at 52 kDa as another study found with the same D2 receptor antibody (Conrad et al., 2010). Membranes were washed (10 min $\times$ 3) in tris-buffered saline tween (TBST), and incubated in IRDye800-conjugated anti-rabbit secondary antibody (1:5000; 611-732-127, Rockland Immunochemicals Inc., Gilbertsville, PA) and visualized using Odyssey Infrared Imaging System (Li-Cor Biosciences, Inc, Lincoln, NE). Actin served as a loading control, and membranes were blocked for 1 hour with NFDm at RT, prior to incubation with primary polyclonal antibody (mouse anti-actin 1:2000, MAB1501R, Millipore) at 4°C for 15 hrs. After washing in TBST, the membranes were incubated in IRDye800-conjugated anti-mouse secondary antibody (610-132-121, 1:5000, Rockland Immunochemicals Inc.) and visualized. Density of all dopamine receptor bands was normalized to actin.

2.6 Data Analysis

Separate Grubbs' tests were applied to all data sets (behavioral and receptor density measures) to identify outliers (Burke et al., 2010; Lowry et al., 2001). Locomotor counts in the novel environment (day one of acclimation to the CPP apparatus) were then analyzed across time using a two-way repeated measures ANOVA followed by Student-Neuman-Keuls (SNK) tests for multiple comparisons, or total locomotion in the 30 minute session was analyzed using one-way ANOVA.

For the CPP experiment, a CPP ratio was used so that the time spent in the paired chamber following conditioning was compared to that prior to conditioning (e.g. Budygin et al., 2004; Schneider et al., 2010). This normalizes individual variability in inter-compartmental activity across rats to reveal the augmentation in time spent in the paired chamber following amphetamine conditioning. Specifically, the CPP ratio was calculated by the difference of the time spent in the amphetamine-paired side (saline-paired side for drug control groups) post-conditioning minus the time spent in the amphetamine/saline-paired side pre-conditioning, divided by the time spent in the amphetamine/saline-paired side pre-conditioning. Three rats of the 57 that received amphetamine pairings did not show any conditioning (a CPP ratio of at least 0.05) and were removed from the study (Brenhouse et al., 2010). The Grubbs' test was then applied to this data set, which resulted in removal of 4 of a possible 88 data points. Dopamine receptor density measurements were analyzed as a percent change from control saline rats. Both CPP and DA receptor data were analyzed with separate two-way ANOVA for social defeat and foot-shock studies. Significant main effects were further analyzed using SNK tests for multiple comparisons. Separate linear regression analyses were used to assess for potential relationships between the time taken to reach social defeat criterion in adolescence, locomotor counts in the novel environment (day 1 of acclimation), and amphetamine-CPP ratios within each adolescent pre-treatment group (stress or control) from the social defeat and foot-shock experiments. All statistical analyses were conducted using Sigma Stat for Windows v3.0.1a (Systat Software Inc., San Jose, California, USA) with significance levels set at $p < 0.05$.

3.0 Results

3.1 Locomotion and Conditioned Place Preference

When locomotion over time was analyzed during the first acclimation exposure to the CPP apparatus in adulthood for rats that had experienced adolescent social defeat, a significant effect of stress pretreatment ($F(1,58) = 18.741$; $p = 0.014$) and time ($F(5,272) = 22.721$; $p < 0.001$) was observed. Post hoc tests showed that socially defeated rats exhibited a significantly greater degree of locomotion in the novel CPP apparatus during the 5–10 min time period ($p = 0.007$) and the final 25–30 min time period ($p = 0.004$) when compared to their controls (Fig. 1A). Furthermore, total locomotion during the 30 min test was also significantly higher for rats defeated as adolescents compared to their control group ($F(1,56) = 4.585$; $p = 0.037$; Fig. 1A insert). For rats exposed to adolescent foot-shock, there was a significant effect of time ($F(5,197) = 20.461$; $p < 0.001$) and an interaction between pretreatment and time ($F(5,197) = 6.632$; $p < 0.001$) on adult locomotion during CPP acclimation when analyzed across time. Post hoc tests suggested that experience of foot-shock during adolescence reduced locomotion during only the first 5 min ($p < 0.001$) of novel CPP exposure when compared to controls (Fig. 1B). However, total locomotion during the 30 min test did not differ between rats exposed to foot-shock and controls ($F(1,42) = 0.001$; $p = 0.991$; Fig. 1B insert).

When amphetamine CPP data was analyzed (Fig. 2A & 2B), two-way ANOVA revealed a significant effect of drug treatment for both the social defeat experiment ($F(1,41) = 83.655$;

$p < 0.001$) and the foot-shock experiment ($F(1,31) = 51.923$; $p < 0.001$). Post hoc tests showed an increase in CPP ratio in rats treated with amphetamine compared to saline groups in both social defeat and foot-shock experiments ($p < 0.001$), suggesting that amphetamine conditioning resulted in preference for amphetamine-paired cues (Figs. 2A & 2B). There was no significant difference in CPP ratios among saline-treated groups ($p > 0.05$). Interestingly, an effect of adolescent stress pretreatment was only apparent following social defeat ($F(1,41) = 8.948$; $p = 0.005$) but not foot-shock stress ($F(1,31) = 0.447$; $p = 0.509$). Post hoc tests revealed that experience of adolescent social defeat and adult amphetamine treatment increased CPP ratio compared to non-defeated controls that also received amphetamine (Fig. 2A, $p = 0.027$). Individual CPP ratios were subsequently plotted to illustrate trends in amphetamine-CPP variance within social defeat and foot-shock groups (Fig. 2C & 2D). Controls that received amphetamine in both the social defeat and foot-shock experiment exhibited either low or high CPP profiles, with distinct separation between these (Fig. 2C & 2D). However, the experience of social defeat in adolescence appeared to shift CPP ratios toward the high preference profile (Fig. 2C). This effect was not observed in rats that experienced foot-shock in adolescence (Fig 2D).

For rats that were socially defeated in adolescence, a positive correlation was found between locomotion in the novel CPP apparatus on day one of acclimation and the degree of amphetamine-CPP ratio (Fig. 3A, $r^2 = 0.458$; $p = 0.022$). However, rats that experienced foot-shock in adolescence exhibited no such correlation (Fig. 3B, $r^2 = 0.046$; $p = 0.550$), and amphetamine-treated controls for each of the social defeat and foot-shock groups exhibited no significant correlation between novelty-induced locomotion and later amphetamine CPP ratio ($p > 0.05$; data not shown). Furthermore, the latency to reach social defeat criterion in adolescent did not significantly correlate with later novelty-induced locomotion or CPP ratio ($p > 0.05$, data not shown).

3.2 Dopamine Receptor Protein Expression

Separate two-way ANOVA revealed no alterations to D1 receptor expression in any brain region sampled following adolescent social defeat and subsequent adult amphetamine conditioning (Table 1). Similarly, there were no changes to D2 receptor expression in the mPFC, NAc shell, and striatum in the social defeat experiment (Table 1). However, in the NAc core there was a significant interaction between adolescent social defeat and amphetamine treatment ($F(1,38) = 4.704$; $p = 0.036$). Pairwise post hoc analysis revealed that within saline treated rats, there was no significant difference in D2 receptor expression between non-stressed controls and rats exposed to social defeat in adolescence ($p > 0.05$). However, non-stressed amphetamine-treated rats had lower NAc core D2 protein levels as compared to non-stressed saline controls ($p = 0.045$; Table 1), indicating that amphetamine conditioning reduced D2 receptor expression in non-stressed rats. Furthermore, socially defeated rats exhibited significantly greater D2 receptor protein content than controls that also received amphetamine ($p = 0.010$; Table 1), but were not significantly different from socially-defeated rats that received saline ($p > 0.05$; Table 1), suggesting that the adolescent stressor prevented the later amphetamine-induced reduction in NAc D2 receptor expression.

There were significant effects of adolescent foot-shock and later amphetamine treatment on D1 and D2 receptors (Table 2). For striatal D1 receptors, there was a significant interaction between adolescent foot-shock stress and amphetamine treatment ($F(1,30) = 5.158$; $p = 0.030$; Table 2). Amphetamine-treated non-stressed rats exhibited lower striatal D1 receptor protein levels compared to non-stressed saline controls ($p = 0.020$; Table 2), suggesting that amphetamine exposure reduced D1 receptor expression in the striatum in this experiment. Saline-treated rats exposed to foot-shock in adolescence also exhibited significantly lower D1 receptor levels in the striatum compared to non-stressed saline treated rats ($p = 0.023$; Table 2), implying that adolescent foot-shock exposure in the absence of later amphetamine

treatment reduces adult D1 receptor expression in this region. In addition, there was a significant main effect of amphetamine on D2 receptor expression in the NAc core in the foot-shock experiment ($F(1,22) = 9.730$; $p = 0.005$; Table 2), although no effect of stress ($F(1,22) = 1.035$; $p = 0.320$) or an interaction between stress and amphetamine was observed ($F(1,22) = 2.156$; $p = 0.156$). This indicates that regardless of stress pre-treatment, amphetamine administration was associated with decreased D2 receptor expression in the NAc core (Table 2).

4.0 Discussion

This study is the first to investigate the effects of social defeat in adolescence on adult conditioned place preference. Most notably, these data suggest that adolescent social defeat stress, but not mild foot-shock stress, increases preference for amphetamine-paired cues in adulthood. In addition, we observed increased locomotion in the novel CPP apparatus following adolescent social defeat stress. Individual locomotion in the novel environment was correlated with the degree of amphetamine CPP in rats exposed to adolescent social defeat, but not adolescent foot-shock. We also investigated D1 and D2 DA receptors following adolescent stress and adult conditioned place preference training, and found that amphetamine conditioning reduced D2 receptor protein content in the NAc core. However, social defeat in adolescence blocked this amphetamine-induced decrease in adult NAc core D2 receptors, while adolescent foot-shock stress did not. Dopamine D1 receptors were altered in the striatum in the foot-shock experiment only. Exposure to foot-shock in adolescence reduced adult striatal D1 receptors, as did amphetamine conditioning in the absence of foot-shock. Overall, these results suggest differential behavioral and neural consequences of adult amphetamine conditioning as related to the type of adolescent stress experience..

Increased locomotion in the novel CPP apparatus following adolescent defeat is similar to previous reports that adult male rats exposed to social defeat or social disruption in adolescence show increased locomotion in a novel open field and an elevated plus maze (McCormick et al., 2008, Watt et al., 2009, Burke et al., 2010), suggesting that social stress in adolescence may reduce acclimation to novel environments. Rats that exhibit heightened locomotion in a novel environment (termed high responders) also exhibit increased amphetamine and cocaine responses (Piazza et al., 1989, Hooks et al., 1991b). Most relevant here, high novelty seeking and hyperactivity in a novel environment predict greater amphetamine CPP in rats (Kleba and Bardo, 1999, Orsini et al., 2004). The neurochemical basis for this relationship has yet to be fully established. However, blunted DA responses in the mPFC and exaggerated NAc DA at basal conditions and in response to cocaine appear to characterize high-responding rats (Hooks et al., 1991b, Piazza et al., 1991, Hooks et al., 1992a). Similar to high responder rats, adult rats that were defeated in adolescence show a blunted DA response in the mPFC and exaggerated NAc core DA response to amphetamine (Burke et al., 2010). Furthermore, in the current social defeat experiment, the level of locomotion in the novel CPP apparatus predicted the degree of amphetamine CPP. Similarly, others have found a positive correlation between locomotion in an open field and the sensitized locomotion response to the same concentration of amphetamine used here (1 mg/kg) (Hooks et al., 1992b). Finally, the distribution of individual amphetamine CPP ratios in stress control groups clearly delineates high- and low CPP responders. However, adolescent social defeat appears to shift the profile of this group to high-CPP responders. Therefore, our findings combined with previous reports suggest that social defeat in adolescence may cause high responder characteristics to manifest in early adulthood.

Our finding that social defeat in adolescent increases preference for amphetamine-paired cues in rats is in line with several human studies which suggest adolescent bullying and

victimization are associated with the later onset of substance abuse related behaviors (Nelson et al., 1995, Hoffmann et al., 2000, Sullivan et al., 2006, Tharp-Taylor et al., 2009, Topper et al., 2010). However, other preclinical reports investigating the effects of social stress in adolescence on adult psychostimulant responses in animal models have not always shown a positive relationship between adolescent social stress and adult drug behaviors. For example, repeated social defeat of hamsters in adolescence failed to alter cocaine-induced locomotion in adulthood (Trzcinska et al., 2002). This implies either species differences in the impact of adolescent social defeat, or differences between cocaine and amphetamine studies. Furthermore, unpredictable social stress in adolescence reduced adult behavioral sensitization to amphetamine (Kabbaj et al., 2002), which may conflict with the current amphetamine CPP data because different behavioral responses to amphetamine were measured in adulthood following differing social stress protocols in adolescence. Also, Mathews et al. (2008) reported that social isolation and rehousing stress in adolescent male and female rats failed to alter amphetamine (0.5 mg/kg) CPP in adulthood. Therefore, social defeat during adolescence appears to have different outcomes on adult amphetamine responses from adolescent exposure to variable social stress including isolation/re-housing

In contrast to social defeat, foot-shock stress in adolescence did not alter adult amphetamine CPP, suggesting social stress has a greater impact on later drug behaviors. However, physical stress in adolescence (bright light, cold, and noise) has been shown to increase preference for cocaine in a two-bottle choice test (Marquardt et al., 2004). One possible explanation for the difference from our current results is that Marquardt et al. (2004) administered the physical stress earlier and for a longer duration (P30-P40) compared to our administration of adolescent foot-shock in the current experiment (P35-P39). Like the current study, direct comparison of social and non-social stressors by others demonstrate that social stress in adolescence often has different outcomes from non-social stressors. For example, unpredictable social stress in adolescence reduced adult behavioral sensitization to amphetamine, while the non-social physical stress during adolescence had no effect on adult sensitization (Kabbaj et al., 2002). A greater influence of social stress versus physical stress could be attributed to the finding that that social defeat stress elicits a larger corticosterone stress response than most other stressors, including foot-shock (Koolhaas et al., 1997). Adolescent social defeat increases plasma corticosterone, and adolescent rats actually exhibit sensitized corticosterone responses to repeated defeat experiences when the defeat is initiated at P35 (such as the current study) but not when initiated in later in adolescence, such as P45 (Watt et al., 2009; Bingham et al 2011). Stress-induced corticosterone responses during adolescence are typically of greater duration than those observed in adulthood, and have been proposed to have enduring effects on brain development and subsequent behavior (McCormick and Mathews, 2007). As such, the sensitized corticosterone response to repeated defeat in adolescence (Watt et al., 2009) may contribute to the altered behavioral and corticosterone responses to amphetamine seen in adulthood (current study and Burke et al., 2010). Overall, the role of corticosterone requires further study as a potential mechanism for the differential effects of adolescent social defeat versus foot-shock stress on adult amphetamine CPP.

Interestingly, the experience of foot-shock in adolescence did cause a decrease in locomotion during the first 5 minutes of adult exposure to the novel CPP apparatus, although there was no effect of previous foot-shock experience on the total locomotion count for the 30 test. We suspect that the previously foot-shocked rats spent some time immobile at the beginning of the test because the floor of one CPP compartment consisted of bars identical to those in the foot-shock apparatus. After 5 minutes had passed without presentation of foot-shock, the rats resumed normal locomotor activity for the remainder of the test.

To our knowledge, this is the first investigation of changes to DA receptors following CPP training. We found that repeated pairings of amphetamine with distinct contextual cues resulted in a reduction in D2 receptors in the NAc core of non-stress control groups compared to non-stress groups that received saline. Repeated psychostimulant injections in the absence of the CPP training also appear to decrease D2 receptor expression in the NAc (Chen et al., 1999), although it appears that 24 or more hours of withdrawal is required to observe this effect (Goeders and Kuhar, 1987, Kleven et al., 1990, Peris et al., 1990). While the previous and current studies did not distinguish between pre- and post-synaptic D2 receptor expression, it could be speculated that the reduction in D2 receptors represents a decrease in the number of the post-synaptic D2 receptors in the NAc as an adaptive response to repeated amphetamine-induced increases in synaptic DA. Interestingly, the effect of amphetamine on D2 receptors in the current study was specific to the NAc core, with no effect on D2 receptor expression in the dorsal striatum, mPFC or NAc shell. In contrast, clinical studies reported that D2 receptors in the striatum are reduced among human substance abusers (reviewed in Volkow et al., 2004). However, the short exposure to amphetamine in the current experiment is much less than that characterizing substance abuse. Therefore, administering amphetamine for a longer time period may yield a reduction in D2 receptor expression within other DA-rich regions such as the striatum, an area thought to underlie compulsive drug taking behaviors after excessive drug exposure (reviewed in Everitt et al., 2008).

The finding that changes to D2 receptor expression were restricted to the NAc core complements our previous report that adolescent defeat was associated with an enhanced adult DA response to amphetamine only in this region, with no alterations seen in the NAc shell or striatum (Burke et al., 2010). Similarly, exposure to a cocaine-paired environment causes an increase in NAc dopamine release measured predominately from the core (Duvauchelle et al., 2000). Furthermore, lesions to the core, but not the shell, impair approach toward a reward associated cue (Parkinson et al., 2000) and decrease conditioned locomotion within an amphetamine-paired chamber (Sellings and Clarke, 2006). In addition, it has been argued that the core, compared to the shell, is more important for eliciting the proper motor response toward an environment paired with reward (Meredith et al., 2008) and that the core is more important for the early phases of drug exposure and acquisition of psychostimulant seeking (Ito et al., 2000, Everitt et al., 2008). Thus, alterations to DA signaling in the NAc core in response to amphetamine, such as increased DA (Burke et al., 2010) and a lack of reduction in D2 receptor expression (current study), could underlie the increased amphetamine CPP behavior observed in adult rats exposed to adolescent social defeat.

The mechanisms by which social defeat stress in adolescence could prevent amphetamine-induced reductions in NAc D2 receptors in adulthood are unknown. However, it is likely that long-lasting alterations to stress-sensitive systems that modulate NAc core D2 expression or DA function in this region may be responsible for the observed results. For example, GDNF-inducible transcription factor (GIF) is up-regulated by corticosterone (Lee et al., 2009) and negatively regulates D2 receptor gene expression (Yajima et al., 1997). We reported previously that adult rats show reduced amphetamine-induced corticosterone responses following adolescent social defeat (Burke et al., 2010). Therefore, reduced amphetamine-induced corticosterone in previously defeated rats may attenuate GIF-induced reduction in D2 receptor gene expression.

In contrast to D2 receptors, we found that D1 receptors were reduced in the striatum, but not the NAc, following repeated amphetamine treatment in the absence of stress in the foot-shock experiment. Previous studies observe decreased D1 receptors in the striatum after both 20 minutes and 2 weeks withdrawal from repeated cocaine (Kleven et al., 1990, Farfel et al.,

1992), giving some precedence for psychostimulant-induced reductions in D1 receptors within the striatum. However, amphetamine did not reduce D1 receptor expression in the striatum of non-stress controls for the social defeat experiment, suggesting that this effect of amphetamine requires replication. Furthermore, there was no effect of social defeat on D1 receptor expression either alone or in combination with amphetamine CPP, whereas foot-shock stress reduced striatal D1 receptor expression in the absence of amphetamine. Together, these data imply that differences in striatal D1 receptor expression are likely not responsible for different behavioral outcomes elicited by adolescent social defeat versus foot-shock stress on amphetamine CPP in adulthood.

Enhanced amphetamine CPP following adolescent social defeat observed in the current study is in agreement with observations that rodents exposed to social defeat in adulthood exhibit increases amphetamine- or cocaine-elicited locomotion and self-administration (Haney et al., 1995; Miczek et al., 2004; Covington & Miczek, 2001; 2005; Quadros and Miczek, 2009; Boyson et al., 2011; Cruz et al., 2001). Furthermore, like adolescent social defeat, the effects of adult social defeat on psychostimulant responses persist for weeks following the stressor (e.g. Miczek et al., 2004; Quadros and Miczek, 2009; Boyson et al., 2011; Cruz et al., 2001). The specific effects of adult social defeat on psychostimulant CPP have not been examined as thoroughly as locomotor and self-administration responses. However, McLaughlin et al (2006) show that social defeat of adult mice enhances cocaine CPP, although the persistence of this effect has not been examined to date. Overall, it appears that the effects of social defeat on behavioral responses to psychostimulants are not age-specific and can be persistent, but the mechanisms that underlie defeat-induced enhancement of psychomotor responses may differ between adolescent and adult defeat. Down-regulation of D2 receptors in the striatum is suggested to mediate adult defeat-induced enhancement of amphetamine-induced locomotion (Dietz et al., 2008). In contrast, the current study finds no role for striatal D2 receptor expression in the enhanced amphetamine CPP expressed following adolescent social defeat. Instead, our results implicate a lack of amphetamine-induced down-regulation of D2 expression in the NAc core as a potential mechanism for enhanced amphetamine CPP in rats exposed to adolescent social defeat. Clearly, studies directly comparing the neurochemical and molecular impact of adolescent and adult social defeat following psychostimulant exposure will clarify this issue.

In conclusion, our results suggest that adolescent social defeat stress increases preference for amphetamine-paired cues in early adulthood, while adolescent foot-shock stress has no effect on this measure. This enhancement in amphetamine CPP in adulthood appears to be related to altered DA function in the NAc core, which warrants more direct investigation. Overall, this rat model provides a useful tool to further elucidate the neural mechanisms that underlie adolescent social stress enhancement of drug-related behaviors in later life (Nelson et al., 1995, Hoffmann et al., 2000, Sullivan et al., 2006, Tharp-Taylor et al., 2009, Topper et al., 2011).

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Abbreviations

CPP	Conditioned place preference
DA	Dopamine

mPFC	Medial prefrontal cortex
NAc	Nucleus accumbens
P	Postnatal day
SNK	Student-Neuman-Keuls

References

- Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse* New York, NY. 2000; 37:167–169.
- Anderson SM, Pierce RC. Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. *Pharmacol Ther.* 2005; 106:389–403. [PubMed: 15922019]
- Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology.* 2000; 153:31–43. [PubMed: 11255927]
- Bingham B, McFadden K, Zhang X, Bhatnagar S, Beck S, Valentino R. Early adolescence as a critical window during which social stress distinctly alters behavior and brain norepinephrine activity. *Neuropsychopharmacology.* 2011; 36:896–909. [PubMed: 21178981]
- Bjorkqvist K. Social defeat as a stressor in humans. *Physiology & behavior.* 2001; 73:435–442. [PubMed: 11438372]
- Blanchard DC, Sakai RR, McEwen B, Weiss SM, Blanchard RJ. Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behavioural brain research.* 1993; 58:113–121. [PubMed: 8136039]
- Boileau I, Dagher A, Leyton M, Welfeld K, Booij L, Diksic M, Benkelfat C. Conditioned dopamine release in humans: a positron emission tomography [¹¹C]raclopride study with amphetamine. *J Neurosci.* 2007; 27:3998–4003. [PubMed: 17428975]
- Brenhouse HC, Dumais K, Andersen SL. Enhancing the salience of dullness: behavioral and pharmacological strategies to facilitate extinction of drug-cue associations in adolescent rats. *Neuroscience.* 2010; 169:628–636. [PubMed: 20639130]
- Boyson C, Miguel T, Quadros I, DeBold J, Miczek K. Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA. *Psychopharmacology.* 2011:1–13.
- Budygin EA, Brodie MS, Sotnikova TD, Mateo Y, John CE, Cry M, Gainetdinov RR, Jones SR. Dissociation of reward and dopamine transporter-mediated properties of amphetamine. *PNAS.* 2004; 101:7751–7786. [PubMed: 15136737]
- Burke AR, Renner KJ, Forster GL, Watt MJ. Adolescent social defeat alters neural, endocrine and behavioral responses to amphetamine in adult male rats. *Brain research.* 2010; 1352:147–156. [PubMed: 20603109]
- Capriles N, Cancela LM. Effect of acute and chronic stress restraint on amphetamine-associated place preference: involvement of dopamine D(1) and D(2) receptors. *European journal of pharmacology.* 1999; 386:127–134. [PubMed: 10618462]
- Chen JC, Su HJ, Huang LI, Hsieh MM. Reductions in binding and functions of D2 dopamine receptors in the rat ventral striatum during amphetamine sensitization. *Life sciences.* 1999; 64:343–354. [PubMed: 10072194]
- Conrad KL, Ford K, Marinelli M, Wolf ME. Dopamine receptor expression and distribution dynamically change in the rat nucleus accumbens after withdrawal from cocaine self-administration. *Neuroscience.* 2010; 169:182–194. [PubMed: 20435100]
- Covington HE 3rd, Miczek KA. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration “binges”. *Psychopharmacology.* 2001; 158:388–398. [PubMed: 11797060]
- Covington HE 3rd, Miczek KA. Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. *Psychopharmacology.* 2005; 183:331–340. [PubMed: 16249907]

- Covington HE 3rd, Tropea TF, Rajadhyaksha AM, Kosofsky BE, Miczek KA. NMDA receptors in the rat VTA: a critical site for social stress to intensify cocaine taking. *Psychopharmacology*. 2008; 197:203–216. [PubMed: 18097654]
- Cruz F, Quadros I, Hogenelst K, Planeta C, Miczek K. Social defeat stress in rats: escalation of cocaine and “speedball” binge self-administration, but not heroin. *Psychopharmacology*. 2011; 215:165–175. [PubMed: 21197616]
- Dietz DM, Dietz KC, Moore S, Ouimet CC, Kabbaj M. Repeated social defeat stress-induced sensitization to the locomotor activating effects of d-amphetamine: role of individual differences. *Psychopharmacology*. 2008; 198:51–62. [PubMed: 18415082]
- Duvauchelle CL, Ikegami A, Castaneda E. Conditioned increases in behavioral activity and accumbens dopamine levels produced by intravenous cocaine. *Behavioral neuroscience*. 2000; 114:1156–1166. [PubMed: 11142647]
- Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW. Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philosophical transactions of the Royal Society of London*. 2008; 363:3125–3135. [PubMed: 18640910]
- Farfel GM, Kleven MS, Woolverton WL, Seiden LS, Perry BD. Effects of repeated injections of cocaine on catecholamine receptor binding sites, dopamine transporter binding sites and behavior in rhesus monkey. *Brain research*. 1992; 578:235–243. [PubMed: 1380862]
- Goeders NE, Kuhar MJ. Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. *Alcohol Drug Res*. 1987; 7:207–216. [PubMed: 2950865]
- Haney M, Maccari S, Le Moal M, Simon H, Piazza PV. Social stress increases the acquisition of cocaine self-administration in male and female rats. *Brain research*. 1995; 698:46–52. [PubMed: 8581502]
- Hoffmann JP, Cerbone FG, Su SS. A growth curve analysis of stress and adolescent drug use. *Substance use & misuse*. 2000; 35:687–716. [PubMed: 10807152]
- Hooks MS, Colvin AC, Juncos JL, Justice JB Jr. Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. *Brain research*. 1992a; 587:306–312. [PubMed: 1525663]
- Hooks MS, Jones GH, Neill DB, Justice JB Jr. Individual differences in amphetamine sensitization: dose-dependent effects. *Pharmacology, biochemistry, and behavior*. 1992b; 41:203–210.
- Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Individual differences in locomotor activity and sensitization. *Pharmacology, biochemistry, and behavior*. 1991a; 38:467–470.
- Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse (New York, NY)*. 1991b; 9:121–128.
- Hooks MS, Juncos JL, Justice JB Jr, Meiergerd SM, Povlock SL, Schenk JO, Kalivas PW. Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *J Neurosci*. 1994; 14:6144–6152. [PubMed: 7931568]
- Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J Neurosci*. 2000; 20:7489–7495. [PubMed: 11007908]
- Kabbaj M, Isgor C, Watson SJ, Akil H. Stress during adolescence alters behavioral sensitization to amphetamine. *Neuroscience*. 2002; 113:395–400. [PubMed: 12127096]
- Klebaue JE, Bardo MT. Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. *Pharmacology, biochemistry, and behavior*. 1999; 63:131–136.
- Kleven MS, Perry BD, Woolverton WL, Seiden LS. Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. *Brain research*. 1990; 532:265–270. [PubMed: 2149299]
- Koolhaas JM, De Boer SF, De Rutter AJ, Meerlo P, Sgoifo A. Social stress in rats and mice. *Acta physiologica Scandinavica*. 1997; 640:69–72.

- Lee SH, Jang MK, Kim OS, Lee OH, Kim NY, Yoo KH, Lee DG, Shong YH, Mouradian MM. Activation of the GDNF-inducible transcription factor (GIF) gene promoter by glucocorticoid and progesterone. *J Steroid Biochem Mol Biol*. 2009; 115:30–35. [PubMed: 19429458]
- Lowry CA, Burke KA, Renner KJ, Moore FL, Orchinik M. Rapid changes in monoamine levels following administration of corticotropin-releasing factor or corticosterone are localized in the dorsomedial hypothalamus. *Hormones and behavior*. 2001; 39:195–205. [PubMed: 11300710]
- Lukkes JL, Mokin MV, Scholl JL, Forster GL. Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Hormones and behavior*. 2009; 55:248–256. [PubMed: 19027017]
- Marquardt AR, Ortiz-Lemos L, Lucion AB, Barros HM. Influence of handling or aversive stimulation during rats' neonatal or adolescence periods on oral cocaine self-administration and cocaine withdrawal. *Behavioural pharmacology*. 2004; 15:403–412. [PubMed: 15343067]
- Mathews IZ, Mills RG, McCormick CM. Chronic social stress in adolescence influenced both amphetamine conditioned place preference and locomotor sensitization. *Developmental psychobiology*. 2008; 50:451–459. [PubMed: 18551462]
- McCormick CM, Mathews IZ. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacology, biochemistry, and behavior*. 2007; 86:220–233.
- McCormick CM, Smith C, Mathews IZ. Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behavioural brain research*. 2008; 187:228–238. [PubMed: 17945360]
- McLaughlin JP, Li S, Valdez J, Chavkin TA, Chavkin C. Social Defeat Stress-Induced Behavioral Responses are Mediated by the Endogenous Kappa Opioid System. *Neuropsychopharmacology*. 2005; 31:1241–1248. [PubMed: 16123746]
- Meredith GE, Baldo BA, Andrezejewski ME, Kelley AE. The structural basis for mapping behavior onto the ventral striatum and its subdivisions. *Brain Struct Funct*. 2008; 213:17–27. [PubMed: 18256852]
- Miczek KA, Covington HE 3rd, Nikulina EM Jr, Hammer RP. Aggression and defeat: persistent effects on cocaine self-administration and gene expression in peptidergic and aminergic mesocorticolimbic circuits. *Neuroscience and biobehavioral reviews*. 2004; 27:787–802. [PubMed: 15019428]
- Miczek, KA.; De Boer, SF. Aggressive, defensive and submissive behavior. In: Wishaw, IQ.; Kolb, B., editors. *The Behaviour of the Laboratory Rat: A Handbook with Tests*. New York: New York: Oxford University Press; 2005. p. 344-352.
- Naha N, Li SP, Yang BC, Park TJ, Kim MO. Time-dependent exposure of nicotine and smoke modulate ultrasubcellular organelle localization of dopamine D1 and D2 receptors in the rat caudate-putamen. *Synapse New York, NY*. 2009; 63:847–854.
- Nansel TR, Overpeck M, Pilla RS, Ruan WJ, Simons-Morton B, Scheidt P. Bullying behaviors among US youth: prevalence and association with psychosocial adjustment. *Jama*. 2001; 285:2094–2100. [PubMed: 11311098]
- Nelson DE, Higginson GK, Grant-Worley JA. Physical abuse among high school students. Prevalence and correlation with other health behaviors. *Archives of pediatrics & adolescent medicine*. 1995; 149:1254–1258. [PubMed: 7581758]
- Orsini C, Buchini F, Piazza PV, Puglisi-Allegra S, Cabib S. Susceptibility to amphetamine-induced place preference is predicted by locomotor response to novelty and amphetamine in the mouse. *Psychopharmacology*. 2004; 172:264–270. [PubMed: 14600800]
- Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ. Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behavioral neuroscience*. 2000; 114:42–63. [PubMed: 10718261]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. San Diego: Academic Press; 1998.
- Peris J, Boyson SJ, Cass WA, Curella P, Dwoskin LP, Larson G, Lin LH, Yasuda RP, Zahniser NR. Persistence of neurochemical changes in dopamine systems after repeated cocaine administration.

- The journal of pharmacology and experimental therapeutics. 1990; 253:38–44. [PubMed: 2329520]
- Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. *Science* New York, NY. 1989; 245:1511–1513.
- Piazza PV, Rouge-Pont F, Deminiere JM, Kharoubi M, Le Moal M, Simon H. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. *Brain research*. 1991; 567:169–174. [PubMed: 1726140]
- Quadros I, Miczek K. Two modes of intense cocaine bingeing: increased persistence after social defeat stress and increased rate of intake due to extended access conditions in rats. *Psychopharmacology*. 2009; 206:109–120. [PubMed: 19513697]
- Schiffer WK, Liebling CN, Reisz C, Hooker JM, Brodie JD, Dewey SL. Cue-induced dopamine release predicts cocaine preference: positron emission tomography studies in freely moving rodents. *J Neurosci*. 2009; 29:6176–6185. [PubMed: 19439595]
- Schneider M, Heise V, Spanagel R. Differential involvement of the opioid receptor antagonist naloxone in motivational and hedonic aspects of reward. *Behav Brain Res*. 2010; 208:446–472.
- Sellings LH, Clarke PB. 6-Hydroxydopamine lesions of nucleus accumbens core abolish amphetamine-induced conditioned activity. *Synapse* New York, NY. 2006; 59:374–377.
- Sullivan TN, Farrell AD, Kliever W. Peer victimization in early adolescence: association between physical and relational victimization and drug use, aggression, and delinquent behaviors among urban middle school students. *Dev Psychopathol*. 2006; 18:119–137. [PubMed: 16478555]
- Sun WL, Zhou L, Quinones-Jenab V, Jenab S. Cocaine effects on dopamine and NMDA receptors interactions in the striatum of Fischer rats. *Brain research bulletin*. 2009; 80:377–381. [PubMed: 19716863]
- Tarazi FI, Baldessarini RJ. Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. *Int J Dev Neurosci*. 2000; 18:29–37. [PubMed: 10708903]
- Tharp-Taylor S, Haviland A, D'Amico EJ. Victimization from mental and physical bullying and substance use in early adolescence. *Addictive behaviors*. 2009; 34:561–567. [PubMed: 19398162]
- Topper LR, Castellanos-Ryan N, Mackie C, Conrod PJ. Adolescent bullying victimisation and alcohol-related problem behaviour mediated by coping drinking motives over a 12 month period. *Addictive behaviors*. 2011; 36:6–13. [PubMed: 20869813]
- Trzcinska M, Bergh J, DeLeon K, Stellar JR, Melloni RH Jr. Social stress does not alter the expression of sensitization to cocaine. *Physiology & behavior*. 2002; 76:457–463. [PubMed: 12126980]
- Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Progress in neurobiology*. 1998; 56:613–672. [PubMed: 9871940]
- Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol*. 2007; 12:227–462. [PubMed: 17678505]
- Unterwald EM, Kreek MJ, Cuntapay M. The frequency of cocaine administration impacts cocaine-induced receptor alterations. *Brain research*. 2001; 900:103–109. [PubMed: 11325352]
- Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Molecular psychiatry*. 2004; 9:557–569. [PubMed: 15098002]
- Watt MJ, Burke AR, Renner KJ, Forster GL. Adolescent male rats exposed to social defeat exhibit altered anxiety behavior and limbic monoamines as adults. *Behavioral neuroscience*. 2009; 123:564–576. [PubMed: 19485563]
- Yajima S, Lammers CH, Lee SH, Hara Y, Mizuno K, Mouradian MM. Cloning and characterization of murine glial cell-derived neurotrophic factor inducible transcription factor (MGIF). *J Neurosci*. 1997; 17:8657–8666. [PubMed: 9348334]

Highlights

- The effects of adolescent stress on drug preferences in adulthood were studied.
- Adolescent social defeat increases adult amphetamine conditioned place preference.
- Adolescent foot-shock does not alter amphetamine conditioned place preference.
- Accumbens core D2 dopamine receptors are reduced by amphetamine place training.
- Adolescent social defeat abolishes amphetamine-induced D2 receptor reductions.

Figure 1.

Locomotion counts (mean $\pm$ SEM) in novel conditioned place preference apparatus during first 30 min exposure. **(A)** Locomotion in adulthood following social defeat in adolescence (N = 30 per group). **(B)** Locomotion in adulthood following foot-shock in adolescence. (N = 22 per group) *Significant difference from control (P < 0.05).

Figure 2.

Conditioned place preference (CPP) following amphetamine conditioning. A positive ratio indicates preference for the amphetamine-paired side and the horizontal line represents the median of the respective control group in scatter plots. Mean ($\pm$ SEM) CPP ratio in adulthood for all treatment groups following either (A) social defeat in adolescence ($N = 10$ – 12 per group) or (B) foot-shock in adolescence ($N = 8$ – 10 per group). #Significant difference from amphetamine-treated rats ($P < 0.05$). *Significant difference between control and social defeat groups ($P < 0.05$). Individual conditioned place preference (CPP) ratios for individuals treated with amphetamine in adulthood from the (C) social defeat experiment ($N = 11$) and (D) the foot-shock experiment ($N = 10$).

Figure 3.

Locomotion in novel conditioned place preference (CPP) apparatus regressed against the CPP ratio for rats exposed to adult amphetamine conditioning following either (A) social defeat in adolescence ($N = 11$) or (B) foot-shock in adolescence ($N = 10$). Regression analysis revealed a significant positive correlation ($P < 0.05$) between novel environment locomotion and CPP ratio for rats exposed to social defeat only.

Table 1

Effects of Adolescent Social Defeat and Adult Amphetamine Conditioning on Dopamine Receptor Expression.

	Control Sal	Control Amp	Defeat Sal	Defeat Amp
<i>D1 dopamine receptor</i>				
mPFC	100.00 ± 5.31	95.33 ± 8.37	96.87 ± 7.18	91.80 ± 7.44
NAc core	100.00 ± 8.19	98.36 ± 5.54	100.76 ± 8.22	110.64 ± 4.96
NAc shell	100.00 ± 4.65	93.36 ± 6.23	97.64 ± 5.84	85.15 ± 7.59
Striatum	100.00 ± 4.17	109.45 ± 6.87	108.86 ± 5.87	96.36 ± 8.78
<i>D2 dopamine receptor</i>				
mPFC	100.00 ± 3.89	85.20 ± 9.15	103.85 ± 7.65	93.43 ± 5.60
NAc core	100.00 ± 2.37	76.05 ± 6.94*	95.63 ± 7.07	105.90 ± 11.53#
NAc shell	100.00 ± 2.23	94.20 ± 8.89	95.78 ± 4.70	96.91 ± 7.49
Striatum	100.00 ± 4.86	82.55 ± 9.04	92.21 ± 4.83	91.57 ± 8.44

Values are a percent change from control saline group.

* Significant difference from control saline group ($p < 0.05$).

Significant difference from control amphetamine group ($p < 0.05$)

Table 2

Effects of Adolescent Foot-shock and Adult Amphetamine Conditioning on Dopamine Receptor Expression.

	Control Sal	Control Amp	Foot-shock Sal	Foot-shock Amp
<i>D1 dopamine receptor</i>				
mPFC	100.00 ± 3.24	116.93 ± 3.40	102.89 ± 5.70	105.32 ± 9.47
NAc core	100.00 ± 6.72	80.61 ± 11.81	88.88 ± 8.63	85.45 ± 5.35
NAc shell	100.00 ± 5.93	97.27 ± 6.63	104.79 ± 5.09	97.85 ± 8.55
Striatum	100.00 ± 3.26	75.00 ± 5.41*	76.03 ± 10.87*	83.33 ± 5.95
<i>D2 dopamine receptor</i>				
mPFC	100.00 ± 3.92	104.28 ± 8.72	106.69 ± 7.80	112.33 ± 6.98
NAc core	100.00 ± 3.93	67.09 ± 9.50 [#]	96.76 ± 7.39	84.92 ± 6.60 [#]
NAc shell	100.00 ± 6.20	115.63 ± 18.30	123.02 ± 20.48	135.59 ± 28.58
Striatum	100.00 ± 1.95	97.43 ± 11.32	105.77 ± 13.29	112.53 ± 11.69

Values are a percent change from control saline group.

* Significant difference from control saline group ($p < 0.05$).[#] Significant main effect of amphetamine irrespective of stress treatment ($p < 0.05$)