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Increased dopamine transporter function as a mechanism for dopamine hypoactivity in the adult infralimbic medial prefrontal cortex following adolescent social stress

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Abstract

Being bullied during adolescence is associated with later mental illnesses characterized by deficits in cognitive tasks mediated by prefrontal cortex (PFC) dopamine (DA). Social defeat of adolescent male rats, as a model of teenage bullying victimization, results in medial PFC (mPFC) dopamine (DA) hypofunction in adulthood that is associated with increased drug seeking and working memory deficits. Increased expression of the DA transporter (DAT) is also seen in the adult infralimbic mPFC following adolescent defeat. We propose the functional consequence of this increased DAT expression is enhanced DA clearance and subsequently decreased infralimbic mPFC DA availability. To test this, *in vivo* chronoamperometry was used to measure changes in accumulation of the DA signal following DAT blockade, with increased DAT-mediated clearance being reflected by lower DA signal accumulation. Previously defeated rats and controls were pre-treated with the norepinephrine transporter (NET) inhibitor desipramine (20mg/kg, ip.) to isolate infralimbic mPFC DA clearance to DAT, then administered the selective DAT inhibitor GBR-12909 (20 or 40mg/kg, sc.). Sole NET inhibition with desipramine produced no differences in DA signal accumulation between defeated rats and controls. However, rats exposed to adolescent social defeat demonstrated decreased DA signal accumulation compared to controls in response to both doses of GBR-12909, indicating greater DAT-mediated clearance of infralimbic mPFC DA. These results suggest that protracted increases in infralimbic mPFC DAT function represent a mechanism by which adolescent social defeat stress produces deficits in adult mPFC DA activity and corresponding behavioral and cognitive dysfunction.

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1. Introduction

Social experiences during development profoundly influence physiology and behavior later in life. This holds true for adolescent bullying victimization, a common yet potent stressor associated with emergence of a wide range of neuropsychiatric disturbances both acutely and in adulthood (Arseneault et al., 2010). The relationship between bullying and later disorders appears to hold true even after controlling for previous psychiatric illness and family environment (Copeland et al., 2013). Effective treatment of these bullying-related disorders would be greatly facilitated if a common underlying neural mechanism could be identified, particularly one amenable to targeting by existing pharmacotherapies. Preclinical research indicates adolescent stress exposure can disrupt the developing medial prefrontal cortex (mPFC) dopamine (DA) system, altering DA neurotransmission to potentiate psychopathology-associated behaviors (Wright et al., 2008; Watt et al., 2014; Burke et al., 2011; Novick et al., 2013). This is also evident from the numerous psychiatric disorders promoted by bullying victimization, which are all characterized by deficits in cognitive function dependent on optimal mPFC DA activity (Robbins and Arnsten, 2009; Testa and Pantelis, 2009). A key regulator of mPFC DA activity is the DA transporter (DAT), which acts to clear synaptic DA and shows functional alterations in psychiatric disorders associated with adolescent bullying (Akil et al., 1999; Krause et al., 2003). Exposure to social aggression in adulthood alters rodent DAT expression, but only in subcortical regions (Filipenko et al., 2001; Lucas et al., 2004). In contrast, rats isolated from weaning show enhanced meosocortical DAT-mediated DA clearance in adulthood compared to those in an enriched environment, suggesting stress exposure encompassing the adolescent period may directly influence later mPFC DAT mechanics (Yates et al., 2012). However, whether adolescent experience of social aggression can similarly alter adult mPFC DAT function is unknown.

Recent research demonstrated that adolescent social defeat in male rats, as a model of teenage bullying, specifically increases DAT expression in the the infralimbic region of the adult mPFC (Novick et al., 2011). This complimented previous studies revealing reductions in adult mPFC DA activity following adolescent social defeat, both basally and in response to amphetamine (Watt et al., 2009, 2014; Burke et al., 2013). Adolescent defeat also causes changes to adult behavior, including heightened locomotion responses to both amphetamine and novelty (Watt et al., 2009; Burke et al., 2013), enhanced seeking of drug-associated cues (Burke et al., 2011), and decreased working memory (Novick et al., 2013), all of which are potentiated by reduced mPFC DA activity (Piazza et al., 1991; Clinton et al., 2006). We hypothesize that the enhanced DAT expression in the infralimbic region of the adult mPFC following adolescent defeat may result in greater DA clearance, reducing availability of extracellular DA to cause deficient mPFC DA activity. Here, we tested this by using *in vivo* chronoamperometry to measure differences in infralimbic mPFC DA signal accumulation in response to DAT blockade. As predicted, adolescent defeat increases DAT function in the adult mPFC, as reflected by lower DA signal accumulation following DAT inhibition. Our findings suggest a mechanistic explanation by which exposure to negative social experiences in adolescence results in deleterious changes to adult behavior and cognition, and may offer a potential treatment target to guide development of more effective pharmacotherapies.

2. Materials and Methods

2.1. Animals

Eighty-one male weanling Sprague-Dawley rats (Postnatal day [P]21) were obtained from the University of South Dakota (USD) Animal Resource Center. All rats were pair-housed according to treatment (defeat or control) and kept at 22°C on a reverse 12-hr light-dark cycle (lights off 10.00). Food and water were available *ad libitum*. Behavioral experiments were conducted between 11:00 and 15:00 under red lighting. All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and received approval from the USD Institutional Animal Care and Use Committee. Every effort was made to minimize the number of animals used and their suffering.

2.2. Adolescent Social Defeat

Adolescent defeat was conducted using a modified resident-intruder paradigm as described previously (Watt et al., 2009). Briefly, at P35 (mid-adolescence, Spear, 2000), male rats were exposed to physical attacks in the cage of a larger, aggressive adult Sprague Dawley resident male rat for 10 min, and were then separated from the resident by a wire mesh barrier for a further 25 min before being returned to the home cage with their cage mate. Adolescents (n = 38) were exposed to social defeat once daily for 5 days (P35 – 39), and were confronted with a different resident male each day to control for variance in defeat intensity. Aged-matched controls (n = 43) did not undergo social defeat, but were placed into a novel empty cage for the duration of the defeat procedure to control for handling and novel environment stress. After the final defeat trial, all rats were left in their original pairs in their home cages and allowed to mature undisturbed to early adulthood (P56).

2.3. Electrochemistry

Extracellular DA levels were measured using *in vivo* chronoamperometry (Blaha and Phillips, 1996; Forster and Blaha, 2003) employing custom made stearate-modified graphite paste electrochemical recording electrodes (recording surface diameter, 200 μm, (Blaha and Jung, 1991)) that allow detection of DA oxidation current without interference from other oxidizable compounds, such as ascorbic acid and norepinephrine (Blaha and Phillips, 1996). Specifically, it has been demonstrated that the addition of stearic acid to the electrodes shifts the oxidation potential of competing analytes to more positive values, allowing distinct resolution of a DA signal (Blaha, 1996). To confirm a distinct and measurable DA oxidation signal, electrodes in the current study were tested *in vitro* by recording voltammetric sweeps (−0.15 V to 0.45 V vs Ag/AgCl, ramp rate 0.01 V/second; Blaha and Jung, 1991) with systematic addition of exogenous ascorbic acid (250 μM), DA (1 μM increments), and norepinephrine (1 μM increments). The signal for DA was found to be between −0.15 and +0.15V, while that for norepinephrine and ascorbic acid were 0.25V–0.35V and over +0.4V, respectively, allowing us to identify these compounds *in vivo* (Fig. 1), and demonstrating selectivity of the electrode for a DA signal.

At P56, rats were anaesthetized with urethane (1.8g/kg, Sigma), a long acting anesthetic that does not affect endogenous DA clearance (Sabeti et al., 2003) Upon cessation of pedal

withdrawal and eye blink reflex, rats were mounted in a stereotaxic frame (David Kopf Inst., CA, USA) with the incisor bar set at −3.3 mm. Body temperature was maintained at 37° C throughout via a heating pad (Harvard Apparatus, Holliston, Massachusetts, USA). The recording electrode was implanted into either the right or left infralimbic mPFC (AP: +3.1, ML: +/− 0.5mm, DV: −4.3 mm from dura; Paxinos and Watson, 1998) in a counterbalanced manner within all treatment groups. The infralimbic mPFC was chosen as this region demonstrated the most robust increase in DAT expression following adolescent defeat (Novick et al., 2011). In addition, ventral regions of the mPFC such as the infralimbic cortex appear to be more reliant on DAT for DA regulation compared to regions located more dorsally (Cass and Gerhardt, 1994). An Ag/AgCl reference electrode was placed in contact with cortical tissue contralateral to the recording electrode, with a stainless steel auxillary electrode fixed to the skull.

Thirty minutes following electrode implantation, an electrometer (Echempro, GMA Technologies, Inc., Vancouver, Canada) was used to evaluate the viability of the graphite paste electrode for DA signal by recording several voltammetric sweeps within the mPFC (−0.15 V to 0.45 V vs Ag/AgCl, ramp rate 0.01 V/second; Blaha and Jung, 1991). Once the voltammetric range of a distinct DA signal was confirmed (Fig. 1), similar to that seen in prior *in vitro* testing, the electrometer was then set to make repetitive chronoamperometric measurements of oxidation current. Specifically, a potential pulse of 1 s duration from −0.15 V to +0.15 V vs Ag/AgCl was applied to the recording electrode every 30 s to ensure selectivity for DA (Fig. 1), with oxidation current being monitored at the end of each 1 s pulse. Thirty minutes of stable baseline measurements were taken prior to all pharmacological manipulations.

2.4. Drug treatment

Accumulation of mPFC DA signal served as a measure of DAT function, since increased transporter-mediated clearance is reflected by lower levels of neurotransmitter accumulation following transporter inhibition (Pozzi et al., 1994). Previously defeated and control rats were assigned to four experimental treatments (Table 1). To isolate DA responses to DAT inhibition within the mPFC, rats were pretreated with either vehicle or the norepinephrine transporter (NET) blocker desipramine (DMI, 20mg/kg, ip; Pozzi et al., 1994), as NET serves as a major DA re-uptake mechanism in this brain region (Pozzi et al., 1994). This dose of DMI is approximately one order of magnitude greater than the ED50 for NET inhibition and so produces near maximal saturation of NET in the mPFC (Iyengar et al., 2004). Ten minutes after DMI, rats were administered either vehicle or the selective DAT inhibitor GBR-12909 (20mg/kg or 40mg/kg, sc.). The 20mg/kg dose GBR-12909 dose is known to increase DA levels in the mPFC to a greater extent than DMI alone (Pozzi et al., 1994), yet is slightly less than the ED50 for DAT inhibition in the brain (26 mg/kg ip., (Andersen, 1989)). The higher 40mg/kg dose represents the top range of GBR-12909 doses used experimentally (Nissbrandt et al., 1991), as higher doses usually cause mortality (Rothman et al., 1991). A group administered GBR-12909 alone was not included, given that this treatment does not raise DA levels in the mPFC (Pozzi et al., 1994; Mazei, et al., 2002; Morón et al., 2002) unless NET is inhibited concurrently (Pozzi et al., 1994). Chronoamperometric measurements were continued for 60 minutes following initial

injection of DMI or vehicle. Rats were then euthanized with a lethal dose of Fatal-plus (1.0 ml, ip. Vortech, Dearborn, MI, USA).

2.5. Histology

Immediately following euthanasia, brains were removed and fixed in 10% formalin prior to sectioning (60 μM). Recording electrode placement was confirmed by two separate experimenters. Only rats with infralimbic mPFC electrode placement were used for subsequent data analysis.

2.6. Drugs

Desipramine HCl (Sigma Aldrich) was dissolved in H_2O at 10mg/ml, with GBR-12909 (Sigma Aldrich) dissolved in a 1:1 solution of water and DMSO. Lower doses (20 mg/kg) of GBR-12909 were prepared at 10mg/ml, while 40mg/kg injections were dissolved in 20mg/ml in order to maintain consistent injection volumes across treatment groups. Desipramine solutions were made fresh daily, while prepared aliquots of GBR-12909 were stored at −20° C for no longer than 14 days.

2.7. Data Analysis

Baseline recordings were normalized to zero current values, with changes following drug or vehicle administration presented as absolute changes in DA oxidation current (Forster and Blaha, 2003). Recordings were then collapsed into ten minute bins, with separate two-way mixed model ANOVA (adolescent stress x repeated measure of time) used to compare DA responses across time between previously defeated rats and controls within each treatment regimen (vehicle + vehicle, DMI + vehicle, DMI + GBR-12909 20mg/kg, and DMI + GBR-12909 40mg/kg). Significant main effects of adolescent stress or interactions were followed by Student-Neuman-Keuls (SNK) tests for multiple comparisons. Any significant main effect of time within each drug treatment group was followed by separate one-way repeated measures ANOVA for either controls or defeated rats. Post-hoc Holm-Sidak tests for multiple comparisons were then used to identify significant changes across time as compared to the zero time point within each group.

To compare the magnitude of drug-induced changes to DA levels, area under the curve (AUC) was calculated for all treatment groups and compared using two-way ANOVA (adolescent stress x drug treatment), followed by post-hoc SNK tests where applicable. A significant main effect of drug treatment was further analyzed by separate one-way ANOVA for each adolescent stress group followed by post-hoc SNK tests. All statistical tests were conducted using SigmaPlot v11.0 (Systat Software Inc., San Jose, California) with alpha level set *a prioi* at 0.05 throughout.

3. Results

The electrode recording surfaces ranged in location between 2.7mm and 3.2mm anterior to bregma and were placed within the entire mediolateral and dorsoventral aspects of the infralimbic region of the mPFC across all subjects (Fig. 1). There were no differences in

electrode placement either between control and defeated rats (Fig. 1), or among drug treatment groups.

Within the vehicle (H_2O) plus vehicle (DMSO/H_2O 1:1) treatment groups (Fig. 2A), there was only a significant main effect of time ($F_{9,126} = 3.794$, $P < 0.001$), but no main effect of either adolescent stress or a stress x time interaction. Subsequent one-way repeated measures ANOVA revealed that an increase in DA signal across time was evident just in the control group ($F_{9,63} = 7.228$, $P < 0.001$) from 30 minutes through 60 minutes (Holm-Sidak, $P < 0.02$), and was likely due to an upward drift in baseline. However, a similar upward drift in baseline across time did not reach statistical significance in previously defeated rats given vehicle plus vehicle ($F_{9,63} = 0.977$, $P = 0.467$).

Following sole NET blockade with DMI (Fig. 2B), there was only a significant effect of time ($F_{9,180} = 12.289$, $P < 0.001$). One-way repeated measures ANOVA showed both defeated and control rats demonstrated an increase in DA signal accumulation across time following DMI plus vehicle treatment ($F_{9,99} = 8.747$, $P < 0.001$ and $F_{9,81} = 4.561$, $P < 0.001$, respectively) with significant increases at 20 minutes and beyond in control rats (Holm-Sidak, $P < 0.001$) and at 30 minutes and beyond in defeated rats (Holm-Sidak, $P < 0.001$).

For animals injected with 20mg/kg GBR-12909 following DMI treatment (Fig. 2C), there were significant main effects of adolescent stress ($F_{1,207} = 5.348$, $P = 0.03$), time ($F_{9,207} = 33.968$, $P < 0.001$), and a stress x time interaction ($F_{9,207} = 3.783$, $P < 0.001$). Both controls and previously defeated rats demonstrated significant increases in DA signal accumulation over time ($F_{9,117} = 35.328$, $P < 0.001$ for controls and $F_{9,90} = 8.80$, $P < 0.001$ for defeated rats), which began at 20 minutes and persisted throughout the experiment (Holm-Sidak, $P < 0.01$). However, pairwise comparisons at individual time points revealed that defeated rats exhibited a significantly lower DA signal than controls at all time points from 30 minutes onward (SNK, $P < 0.05$).

The 40mg/kg dose of GBR-12909 produced a similar pattern of results to that elicited by 20mg/kg (Fig. 2D), with significant main effects of adolescent stress ($F_{1,171} = 5.761$, $P = 0.027$), time ($F_{9,171} = 45.113$, $P < 0.001$), and a stress x time interaction ($F_{9,171} = 2.667$, $P = 0.006$). Both controls and defeated rats treated with 40mg/kg GBR-12909 showed significant increases in DA signal accumulation across time ($F_{9,90} = 21.040$, $P < 0.001$ for controls and $F_{9,81} = 21.501$, $P < 0.001$ for defeated rats), which began at 20 minutes and remained high throughout (Holm-Sidak, $P < 0.001$). However, previously defeated rats showed significantly less DA signal accumulation than controls at all time points from 20 minutes onwards (SNK, $P < 0.05$).

When the magnitude of infralimbic mPFC DA signal accumulation (calculated as AUC) was compared across groups (Fig. 4), there was a significant effect of both adolescent stress ($F_{1,73} = 7.970$, $P = 0.006$) and drug treatment ($F_{3,73} = 9.598$, $P < 0.001$) but no interaction ($F_{3,73} = 1.477$, $P = 0.228$). Within control rats, one way ANOVA ($F_{3,39} = 8.423$, $P < 0.001$) revealed that all drug treatments increased DA signal accumulation compared to vehicle plus vehicle (SNK, $P < 0.05$). Administration of DMI combined with either 20 or 40 mg/kg

GBR-12909 elicited significantly greater total DA accumulation than DMI alone in control rats (SNK, $P < 0.05$; Fig. 4), but there was no difference between the effects of either dose of GBR-12909 (SNK, $P = 0.388$; Fig. 4). Defeated rats also exhibited significantly total greater DA signal accumulation following drug administration (one-way ANOVA, $F_{3,34} = 3.036$, $P < 0.05$), with all drug treatments increasing DA accumulation compared to vehicle plus vehicle (SNK, $P < 0.05$; Fig. 4). However, unlike for control rats, DMI combined with either 20 or 40 mg/kg GBR-12909 did not increase total DA accumulation beyond the effects of DMI alone in previously defeated subjects (SNK, $P > 0.844$; Fig. 4). Administration of 20 mg/kg GBR-12909 produced an effect equivalent to that of 40 mg/kg GBR-12909 in the defeated group (SNK, $P = 0.954$; Fig. 4). While the lack of statistical interaction in the initial two-way ANOVA precluded any direct comparison of the total DA accumulation response to each drug treatment between stress groups, there was a clear increase in the response of control rats to the combined DMI+GRB-12909 treatment compared to DMI alone, suggesting an effect specific to DAT inhibition that was absent in social defeat subjects (Fig. 4).

4. Discussion

Experience of social defeat in adolescence increased DAT function in the adult infralimbic mPFC, as reflected by less DA signal accumulation using chronoamperometric measurement following DAT blockade. Specifically, previously defeated rats receiving a combination of DMI plus GBR-12909 (20mg/kg and 40mg/kg) exhibited significantly lower DA accumulation across time compared to controls, suggesting that neither dose of DAT inhibitor was able to saturate the higher levels of adult infralimbic mPFC DAT expression sites observed following adolescent defeat (Novick et al., 2011). The comparable DA accumulation response to DMI plus vehicle treatment in both controls and defeated rats suggests that NET-mediated DA transport in the adult infralimbic mPFC is not altered by adolescent social defeat, and so is unlikely to contribute to the differences observed following DAT blockade with GBR-12909. These results support the hypothesis that increased DAT expression in the adult infralimbic mPFC following adolescent defeat leads to functional increases in DAT-mediated clearance, which in turn may contribute to defeat-induced mPFC dopaminergic hypofunction (Watt et al., 2009, 2014; Burke et al., 2013) by lowering availability of extracellular DA. Increased DA uptake could also enhance end-product inhibition of tyrosine hydroxlase within terminals to directly reduce DA synthesis (Bannon and Roth, 1983) and produce the previously observed decrease in adult mPFC DA content following adolescent defeat (Watt et al., 2009).

It is necessary to point out that our measurements were restricted to the infralimbic mPFC, as only this region of the adult mPFC shows increased DAT expression following adolescent defeat, with no change observed in more dorsal regions such as the prelimbic and cingulate mPFC (Novick et al., 2011). Clearance of DA is most heavily mediated by DAT in ventral regions of the mPFC, which include the infralimbic, compared with regions located more dorsally (Cass and Gerhardt, 1995). Combined with the distinct and often opposing roles of the ventral and dorsal mPFC in regulation of cognitive and emotive behaviors (Dalley et al., 2004; Vidal-Gonzalez et al., 2006; Moorman et al., 2015), it is possible that the effects of adolescent defeat on adult DAT function are specific to the infralimbic mPFC.

Increased adult mPFC DAT functional expression caused by adolescent defeat may reflect a compensatory mechanism to cope with excessive stress-induced DA release. Stressors preferentially activate mesocortical DA release (Abercrombie et al., 1989), which may be augmented during adolescence by enhanced mPFC neuronal responses to stress (Lyss et al., 1999). Adolescent rats exposed to threat of social defeat show increased extracellular DA release in the mPFC (Watt et al., 2014), similar to that shown by adult rats in response to social challenge (Tidey and Miczek, 1996). Such increased mPFC DA release during adolescent defeat would be expected to result in enhanced activation of DA type 2 (D2) autoreceptors located on mPFC DA terminals, which function to inhibit further DA release (Wolf and Roth, 1987) and restore DA homeostasis. Activation of D2 autoreceptors can also increase DAT expression and function (Cass and Gerhardt, 1994; Lee et al., 2007; Owens et al., 2012). Specifically, D2 autoreceptors have been found to increase activity of the nucelar orphan receptor *Nurr1* (Kim et al., 2006), which in turn enhances DAT transcription (Sacchetti et al., 2001). Pharmacologically inhibiting D2 autoreceptors in the mPFC during adolescent defeat prevents later decreases in adult mPFC DA activity (Watt et al., 2014), potentially by blocking direct D2 autoreceptor-mediated increases in DAT functional expression, which is a possibility that requires further study. While increased DAT function induced by stress-induced activation of D2 autoreceptors may be immediately advantageous for facilitating restoration of mPFC DA homeostasis, the fact that it persists well beyond the adolescent defeat experience represents a maladaptive overcompensation that will reduce DA availability in adulthood, just as genetic overexpression of DAT in mice and subsequently enhanced DA clearance results in decreased extracellular DA levels (Salahpour et al., 2008).

While the present study sought measure differences in DAT function, it is possible that other mechanisms besides DAT mediated clearance may have contributed to the decreased DA signal in defeated rats following administration of GBR-12909. For example, decreased release of DA in the mPFC would be expected to lead to lower DA signals in defeated rats, a phenomenon that might be due to differences in release modulation either by D2 autoreceptors or alpha-2 autoreceptors (Devoto et al., 2004; Yamamoto and Novotney, 1998). While previous work did not find any difference in mPFC D2 receptor expression between defeated rats and controls (Burke et al., 2011), an increase in autoreceptor sensitivity remains a distinct possibility for future testing (Watt et al., 2014). That said, both mPFC D2 and alpha-2 autoreceptors are known to decrease the DA response to DMI (Devoto et al., 2004; Yamamoto and Novotney, 1998). In the present study, we found no differences in DA signal when DMI was administered alone, arguing against a role for decreased DA release by mPFC autoreceptors in contributing to the dampened DA accumulation response to DAT blockade in defeated rats. Another possibility is that the dampened DA signal following treatment with GBR-12909 in defeated rats may have been due to enhanced activity at somatodendritic D2 autoreceptors in the ventral tegmental area (VTA), which would be expected to attenuate mPFC DA release. The potential influence of brain regions outside the mPFC on the current results is important to keep in mind given that drugs in the current study were administered systemically. While differences in somatodentritic autoreceptor function influencing the infralimbic mPFC DA signal in defeated rats cannot be ruled out and deserves further study, cortical DA release has been

found to be less sensitive to somatodendritic receptor feedback, at least when compared to DA release in the nucleus accumbens (Chen and Pan, 2000).

In conclusion, the pharmacological results from the present study are suggestive of enhanced DAT functional expression in the infralimbic cortex of adult rats subjected to social defeat in adolescence, providing a potential mechanism for the deficits in adult mPFC DA activity and related behaviors found in these animals. Interventions that target enhanced mPFC DAT function could thus potentially prevent and/or treat the deleterious effects of adolescent social defeat such as increased drug seeking (Burke et al., 2011) and decreased working memory (Novick et al., 2013). For example, preclinical research demonstrates that repeated amphetamine treatment at low doses (1.0 mg/kg) normalizes adult deficits in both behavioral and mPFC DA responses to amphetamine challenge following adolescent social defeat (Burke et al., 2013), which may be related to the reported effect of chronic psychostimulant treatment in decreasing mPFC DAT function (Harvey et al., 2011). Moreover, psychostimulants including amphetamine are routinely given clinically for treatment of attention deficit hyperactivity disorder (ADHD), one of the disorders associated with bullying victimization (Holmberg and Hjern, 2008; Kumpulainen, 2008; Sciberras et al., 2012), and their administration at low doses improves mPFC DA-dependent cognitive task performance in ADHD patients (Arnsten, 2006). Psychostimulants primarily exert their pharmacological effect by blocking catecholamine transporters (NET and DAT; Arnsten, 2006; Fleckenstein et al., 2007). The findings of the current study indicate that enhanced infralimbic DAT function is a consequence of adolescent social stress exposure. Thus, further pre-clinical testing of similar existing pharmacotherapies targeting DAT provides a future research direction for treating disorders of adolescent bullying characterized by mesocortical DA dysfunction.

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Highlights

- Adolescent defeat results in less prefrontal dopamine accumulation upon transporter blockade
- Lower dopamine accumulation suggests greater dopamine transporter function
- Targeting dopamine transporters may rectify negative effects of adolescent stress

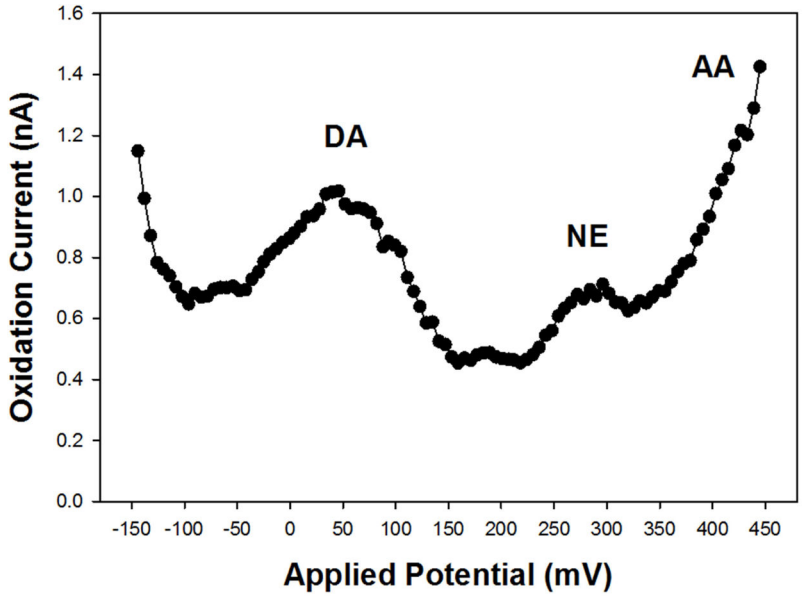

Figure 1.

Example of an *in vivo* voltammetric sweep recorded from the rat infralimbic medial prefrontal cortex using a stearate-modified carbon paste electrode. Identification of peaks was accomplished by prior *in vitro* testing using systematic additions of known compounds to the test solution. Note that stearate-modification of the electrode ensures that the oxidation potentials for norepinephrine (NE) and ascorbic acid (AA) signals are shifted to more positive values that are distinct from the applied potential used to detect dopamine (DA) with chronoamperometry (−150 mV to +150 mV).

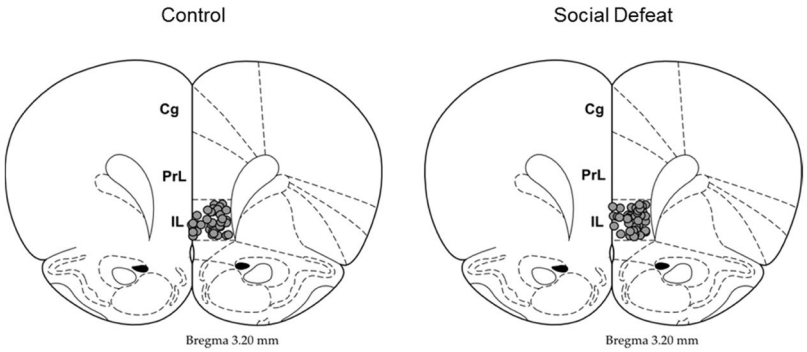

Figure 2.

Representative coronal diagrams of recording electrode tip placement, as represented by shaded circles. For clarity, only left side placements are shown, but placements did not differ between left and right infralimbic cortices. Figure adapted from Paxinos and Watson (1998).

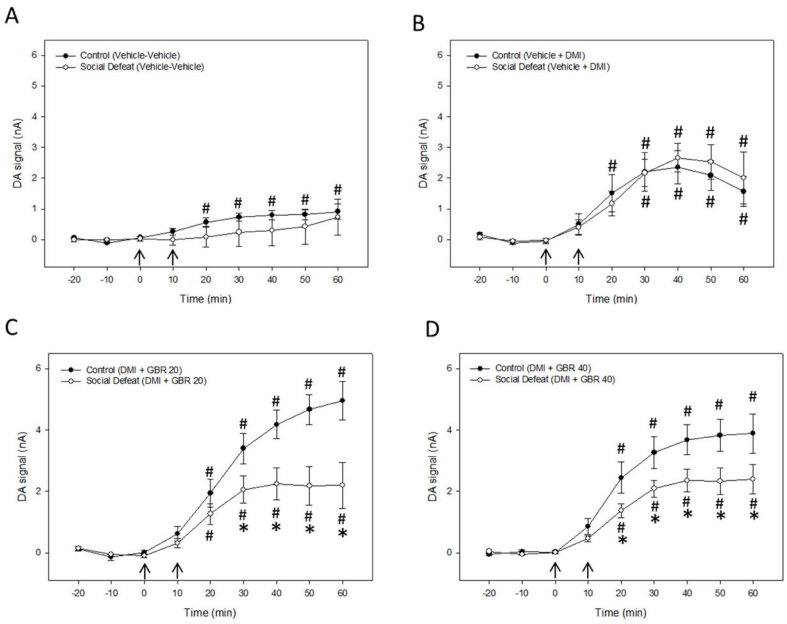

Figure 3.

Effect of systemic DAT inhibition on DA accumulation across time within the adult infralimbic mPFC following adolescent social defeat or control treatment. Data are expressed as mean changes ± SEM in DA oxidation current following administration of **(A)** vehicle plus vehicle **(B)** DMI (20mg/kg) plus vehicle **(C)** DMI (20mg/kg) plus GBR-12909 (20mg/kg) and **(D)** DMI (20mg/kg) plus GBR-12909 (40mg/kg). Arrows represent time points of injection (0 minutes DMI or vehicle ip., 10 minutes GBR-12909 or vehicle sc.). # Significant difference from time point 0 ($P<0.05$), * Significant difference between adolescent stress conditions ($P<0.05$).

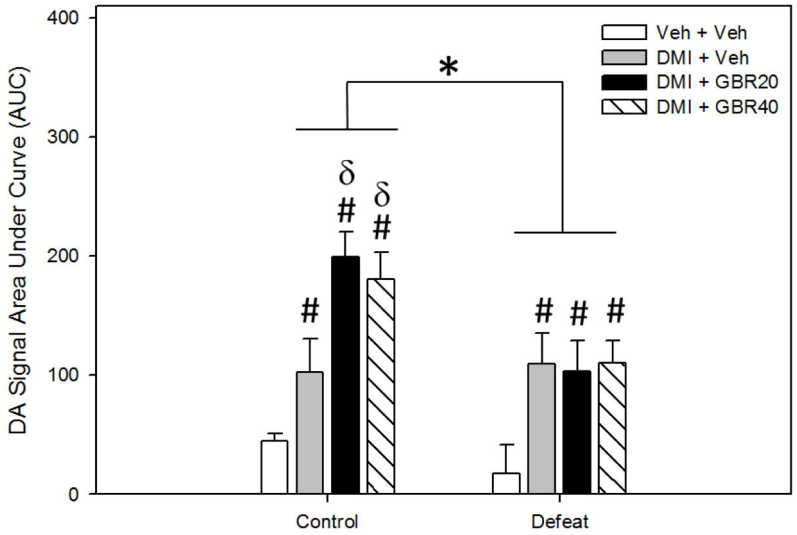

Figure 4.

Magnitude of DA accumulation within the adult infralimbic mPFC in previously defeated rats and controls following systemic NET and DAT inhibition. Data are expressed as mean area under the curve (AUC) ± SEM of changes in DA signal from 0 to 60 minutes following drug treatment. * Significant main effect of stress ($P<0.05$). # Significant difference from vehicle plus vehicle treatment ($P<0.05$), δ significant difference from DMI plus vehicle treatment ($P<0.05$).

Table 1

Assigned experimental treatment groups.

Adolescent stress	Drug pre-treatment (ip.)	Drug treatment (sc.)	N
Control	Vehicle (H_2O)	Vehicle (DMSO)	8
Social defeat	Vehicle (H_2O)	Vehicle (DMSO)	8
Control	Desipramine (20mg/kg)	Vehicle (DMSO)	12
Social defeat	Desipramine (20mg/kg)	Vehicle (DMSO)	9
Control	Desipramine (20mg/kg)	GBR-12909 (20mg/kg)	13
Social defeat	Desipramine (20mg/kg)	GBR-12909 (20mg/kg)	11
Control	Desipramine (20mg/kg)	GBR-12909 (40mg/kg)	10
Social defeat	Desipramine (20mg/kg)	GBR-12909 (40mg/kg)	10