Psychopharmacology (2014) 231:1627–1636
DOI 10.1007/s00213-013-3353-9

ORIGINAL INVESTIGATION

Decreased prefrontal cortex dopamine activity following adolescent social defeat in male rats: role of dopamine D_2 receptors

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Received: 1 June 2013 / Accepted: 26 October 2013 / Published online: 23 November 2013

Abstract
Rationale Adverse social experience in adolescence causes reduced medial prefrontal cortex (mPFC) dopamine (DA) and associated behavioral deficits in early adulthood.
Objective This study aims to determine whether mPFC DA hypofunction following social stress is specific to adolescent experience and if this results from stress-induced DA D_2 receptor activation.
Materials and methods Male rats exposed to repeated social defeat during adolescence or adulthood had mPFC DA activity sampled 17 days later. Separate experiments used freely moving microdialysis to measure mPFC DA release in response to adolescent defeat exposure. At P40, 49 and 56 mPFC DA turnover was assessed to identify when DA activity decreased in relation to the adolescent defeat experience. Finally, nondefeated adolescent rats received repeated intra-mPFC infusions of the D_2 receptor agonist quinpirole, while another adolescent group received intra-mPFC infusions of the D_2 antagonist amisulpride before defeat exposure.
Results Long-term decreases or increases in mPFC DA turnover were observed following adolescent or adult defeat, respectively. Adolescent defeat exposure elicits sustained increases in mPFC DA release, and DA turnover remains elevated beyond the stress experience before declining to levels below normal at P56. Activation of mPFC D_2 receptors in nondefeated adolescents decreases DA activity in a similar manner to that caused by adolescent defeat, while defeat-induced reductions in mPFC DA activity are prevented by D_2 receptor blockade.
Conclusions Both the developing and mature PFC DA systems are vulnerable to social stress, but only adolescent defeat causes DA hypofunction. This appears to result in part from stress-induced activation of mPFC D_2 autoreceptors.

Keywords Adolescence · Stress · Social defeat · Prefrontal cortex · Dopamine · D_2 autoreceptor · Amisulpride · Quinpirole

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Introduction

The prefrontal cortex (PFC) dopamine (DA) system is crucial for mediating cognitive control processes that permit optimal performance during complex cognitive tasks, otherwise known as executive function (Floresco and Magyar 2006; Robbins and Arnsten 2009). The regulatory components of this system undergo substantial changes during postnatal development, particularly during adolescence (Andersen et al. 1997, 2000; Lewis 1997; Spear 2000; Brenhouse et al. 2008). While these changes facilitate the behavioral transition from adolescence to adulthood (Spear 2000; Crone and Dahl 2012), the developing PFC DA system is thus vulnerable to stress-induced disruption, which could result in maladaptive behavioral states later in life.

Many stressors encountered by adolescents are social in nature, as this is a time of increased social contact and learning (Spear 2000; Douglas et al. 2004; Varlinskaya and Spear

2008). The adolescent PFC DA system appears especially sensitive to negative social experience, with male rats exposed to repeated social defeat during mid-adolescence (postnatal day [P] 35–39) showing decreased medial PFC (mPFC) DA activity and content both at baseline and in response to amphetamine as young adults (P56–63; Watt et al. 2009; Burke et al. 2010, 2013). These changes appear specific to the mPFC, with no effect seen in subcortical DA systems such as the nucleus accumbens or striatum (Watt et al. 2009; Novick et al. 2011; Burke et al. 2013). Alterations to adult mPFC activity caused by adolescent defeat are accompanied by behavioral changes reflecting mPFC DA hypofunction and compromised cognitive control, such as increased risk taking in novel mildly anxiogenic environments (Watt et al. 2009), enhanced responses to rewarding psychostimulants and their associated cues (Burke et al. 2011, 2013), and decreased performance in working memory tasks (Novick et al. 2013).

While adolescent social defeat has a negative effect on adult mPFC DA activity, the question remains as to whether this is specific to adolescent experience. Adolescent social instability enhances amphetamine-induced locomotion and fear conditioning deficits in adulthood, effects not apparent when the same stress is applied to adult rats (McCormick et al. 2005; Morrissey et al. 2011). Mediation of psychostimulant-induced behaviors by the mPFC DA system is also more pronounced in adolescence than adulthood (Brenhouse et al. 2008; Mathews and McCormick 2012). The anxiogenic drug beta-carboline FG-7142, which enhances mPFC DA release and turnover, increases mPFC metabolism to greatest effect in adolescent rats (Lyss et al. 1999). Combined, this suggests alterations to mPFC DA activity caused by social defeat will have more profound effects on later DA activity when experienced in adolescence rather than adulthood. This hypothesis was directly tested by the current study.

Social defeat experienced in adolescence results in decreased mPFC DA activity in early adulthood, but by what mechanism? Physical and social stressors cause acute increases in DA release in the mPFC of adult rats (Cenci et al. 1992; Tidey and Miczek 1996), but excessive mPFC DA release can be detrimental to cognitive function (Pani et al. 2000). To restore homeostasis following stress such as social defeat, excessive mPFC DA release may be reduced through activation of presynaptic DA D_2 receptors located on DA terminals (Talmaciu et al. 1986; Wolf and Roth 1987; Ozaki et al. 1989). Activation of these autoreceptors also indirectly reduces DA synthesis in mPFC terminals by enhancing end-product inhibition of tyrosine hydroxylase (Galloway et al. 1986; Wolf et al. 1986; Wolf and Roth 1987). If adolescent social defeat-induced increases in mPFC DA release are similar to those seen in adults, it is possible that decreased mPFC DA release and content following adolescent defeat may partly result from increased mPFC D_2 autoreceptor activity, especially if these receptors continue to remain over-active beyond the stress experience. To test this potential mechanism, the current study examined defeat-induced mPFC DA release during adolescence, and also tested the role of D_2 receptors in mediating later decreases in mPFC DA activity via pharmacological manipulations using drug concentrations shown to exert preferential actions at D_2 autoreceptors.

Methods

Animals

Male Sprague Dawley experimental rats ($n=190$) were purchased from the University of South Dakota (USD) Animal Resource Center at 3 weeks old, and housed in pairs for the entire experiment. Resident adult male rats (300 g or more) were housed individually for at least 3 weeks prior to experiments to encourage territoriality. Rats were maintained at 22 °C on a reverse 12-h light/12-h dark cycle (lights off at 10 am) with free access to food and water. All experimental procedures were performed in the active phase of the light cycle (between 11 am and 3 pm) under red lighting. Procedures were approved by the USD IACUC and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition, 2011). All efforts were made to reduce animal suffering and numbers.

Experiment 1: testing if social defeat effects on mPFC DA activity are specific to adolescent stress

Social defeat procedures Rats experienced five consecutive days of social defeat, as described previously (Watt et al. 2009; Burke et al. 2010). Briefly, at P35 (mid-adolescence, Spear 2000; Andersen 2003) or P70 (adulthood, Spear 2000) male rats ($n=10$/age group) were exposed to social defeat for 10 min in the home cage of a markedly larger adult male rat pre-screened for aggressive behavior. Experimental rats were considered defeated after exhibiting three consecutive submissive postures in response to resident attacks (Watt et al. 2009). Intruders and residents were then separated by a wire mesh barrier for 25 min to prevent further physical contact but allow transmission of auditory, olfactory, and visual cues. Intruder rats were confronted with a different resident male during each trial to control for variance in defeat intensity. Age-matched male controls ($n=10$/age group) were placed in a novel empty cage for the duration of each defeat trial to control for handling and novel environment stress (Covington and Miczek 2005; Vidal et al. 2007; Watt et al. 2009; Burke et al. 2010). Following repeated social defeat or control treatments, the originally paired adolescent rats were left undisturbed in their home cages (except for husbandry) for 17 days until early adulthood (P56). This 17 day period matched that used in our previous studies, in which rats defeated in adolescence (P35–

39) showed reduced DA activity in the mPFC when sampled at P56 (Watt et al. 2009; Burke et al. 2010). The rats defeated in adulthood (P90–94) were treated identically for consistency between the adolescent and adult defeat experiments, and also sampled 17 days after final defeat (P91). Comparisons of defeat intensity (a combined measure of latency and frequency of resident attacks and intruder submissions, along with qualitative measures of intruder vocalizations and bites by residents) demonstrated that defeat experiences were similar between adolescent and adult intruders (data not shown).

Collection and processing of brains At P56 (rats defeated in adolescence) or P91 (rats defeated in adulthood), rats were decapitated and brains rapidly removed, frozen on dry ice and stored at −80 °C. Frozen brains were sliced coronally at 300 μm using a cryostat (Leica Jung CM 1800; North Central Instruments, Plymouth, MN) and sections thaw-mounted on glass slides and stored at −80 °C. The entire mPFC (comprising infralimbic, prelimbic, and cingulate cortices, Paxinos and Watson 1997) was microdissected with a 580-μm id cannula using a dissecting microscope and freezing stage (Physiotemp; North Central Instruments). The tissue was expelled into 60 μl of sodium acetate buffer (pH 5.0) containing 0.1 μM of internal standard (α-methyl-DA), and the cells lysed by freeze-thawing samples (Watt et al. 2009; Burke et al. 2010).

Measurement of DA activity using HPLC-EC The mPFC was analyzed for tissue DA and DOPAC concentrations using high-performance liquid chromatography (HPLC) with electrochemical detection (EC). Details of this assay have been described previously (see Watt et al. 2009; Burke et al. 2010). Concentrations of DA and DOPAC were corrected for recovery using CSW32 v1.4 Chromatography Station for Windows (DataApex, Prague, Czech Republic), and expressed as a ratio of DOPAC/DA as an index of DA turnover (a measure of DA activity, Scholl et al. 2010).

Experiment 2: the effects of social defeat on DA release in the mPFC during adolescence

Surgical procedures At P28, rats were anesthetized with isoflurane and stereotaxically (David Kopf, Tujunga, CA) implanted with a guide cannula (20 gauge; Plastics One, Roanoke, VA, USA) 1 mm above the mPFC (AP, +2.8 mm; ML, ±0.5 mm; DV, −0.2 mm; Paxinos and Watson 1997). Cannulae were fixed to the skull with a combination of glass ionomer cement (GC Corporation, Alsip, IL, USA) and a coating of cranioplastic cement (Plastics One) using dental screws as anchor points. After surgery, rats were injected with the analgesic ketaprofen (5 mg/kg., im.; Met-Vet, Libertyville, IL) and allowed to recover for 6 days before further experimentation.

Social defeat Rats were defeated daily from P35–37 (or exposed to control conditions) as described for Experiment 1. Social controls were used to control for social experience in the absence of defeat by introducing the rats into the resident's cage for 35 min/day from P35–37, but kept separate from the resident by a mesh barrier to prevent physical interactions.

Microdialysis To measure DA release after a number of defeat exposures, microdialysis was conducted during the 4th defeat/control experience (P38). The night before the microdialysis experiment, rats were anesthetized with isoflurane and implanted with a microdialysis probe (3.5 mm membrane length, typical recovery 20 %; Barr and Forster 2011) into the mPFC, to a depth of 4.9 mm from dura. The probe was perfused with artificial cerebrospinal fluid (aCSF) overnight at a rate of 0.4 μl/min using a microinfusion pump (CMA, North Chelmsford, MA, USA) and the rats allowed to recover in a 10-gal glass testing chamber with free access to food and water. Collection of dialysates at 20 min intervals for DA analysis was initiated 12 h after probe implantation. All measurements were conducted during the dark phase of the photoperiod. After three consecutive stable DA baseline samples, rats were either placed into the resident's cage in the absence of the resident or were placed into the home cage of a resident rat with the resident rat behind a mesh barrier (mimicking the second phase of the defeat procedure as described for Experiment 1) to allow the exchange of olfactory and visual cues without the resident rat physically interacting with the experimental intruder. Preliminary trials involving actual defeat failed because resident rats would break the probes or interfere with the microdialysis lines arising from the experimental rats' heads. The defeat group rats ($n=7$) were socially defeated from P35–37 and then exposed to the resident during microdialysis testing (on P38). Rats in the social control group ($n=6$) were not defeated but were exposed to a resident via the mesh barrier during P35–37, and were similarly exposed to the resident during microdialysis. Non-social controls ($n=6$) underwent control procedures on P35–37 and were exposed to an empty home cage of a resident during the microdialysis experiment to control for handling. Rats were returned to the microdialysis test chamber after 20 min of exposure to the resident or resident's cage, and dialysates collected until DA returned to baseline levels. DA was analyzed using HPLC-EC as described previously (for details, see Burke et al. 2013). Voltage output was recorded by Clarity v2.4 Chromatography Station for Windows (DataApex) and DA peaks identified by comparison to a DA standard (11.05 pg/5 μl).

Histology Rats were killed by an overdose of sodium pentobarbital (0.5 ml Fatal Plus, i.p.; Vortech, Dearborn, MI, USA), with brains removed and fixed in 10 % buffered formalin (Fisher Scientific, Fair Lawn, NJ, USA) then frozen and sectioned (60 μm) on a sliding microtome. Probe placement

was evaluated under a light microscope by two experimenters, one blind to treatment.

Experiment 3: timeline for the reduction of mPFC DA activity following adolescent social defeat

Social defeat procedures and DA analysis Rats were exposed to five consecutive days of social defeat or control conditions from P35–39, as described for Experiment 1. Brains were collected either the day following the last social defeat episode (P40), at a midpoint between adolescence and early adulthood (P49) or at early adulthood (P56; $n = 7$–10/group/time point). The mPFC was processed and analyzed for DA activity (turnover) using HPLC-EC as described for Experiment 1.

Experiment 4: the role of D_2 receptors in adolescent defeat-induced decreases in mPFC DA activity

This last experiment tested whether activating D_2 receptors in the mPFC during adolescence would mimic the effects of adolescent social defeat by resulting in reduced DA activity in early adulthood. We also tested if blocking D_2 receptors during adolescent defeat would prevent decreased DA activity in early adulthood. Quinpirole and amisulpride were used to activate or block D_2 receptors, respectively. The doses for each drug were based on previous work demonstrating preferential presynaptic D_2 autoreceptor effects when infused directly into the mPFC or other brain regions (Barik and de Beaurepaire 1996; Doherty and Gratton 1999), as we hypothesized changes to mPFC DA activity following adolescent defeat result partly from stress-induced activation of D_2 autoreceptors.

Surgery and acclimation to infusions At P28, rats were anesthetized and intracranial surgery performed as described for Experiment 2 to implant bilateral mPFC guide cannulae (22 gauge, 2 mm long; Plastics One). After 3 days of recovery, rats were acclimated to handling and infusion procedures for three consecutive days. Rats were gently held in cloth, and 30 gauge infusion cannulae (3 mm longer than the guide) were inserted through the guide cannulae to target the ventral portion of the mPFC, as this region shows changes to DA transporter expression in early adulthood following adolescent social defeat as measured using autoradiography (Novick et al 2011). Infusions of 0.3 μl aCSF bilaterally into the mPFC were done at a rate of 0.3 μl/min using a microinfusion pump (Stoelting, Wood Dale, IL). Cannulae remained in situ for 1 min afterwards to allow diffusion away from the tip.

MPFC quinpirole infusions during adolescence From P35–39, non-defeated rats received daily bilateral mPFC infusions (0.3 μl per side) of vehicle (aCSF) or quinpirole hydrochloride (100 ng/0.3 μl; Doherty and Gratton 1999; Tocris, Minneapolis, MN). Brains were collected either the day after the last infusion (P40, $n = 8$–9/group) or in young adulthood (P56, $n = 8$–9/group) and analyzed for DA activity (turnover) as described for Experiment 1.

MPFC amisulpride pre-treatment during adolescent social defeat From P35–39, rats received bilateral intra-mPFC infusions of either vehicle (aCSF) or amisulpride (50 ng/0.3 μl; Barik and de Beaurepaire 1996; Tocris) 20 min prior to daily defeat or control procedures (see Experiment 1). Brains were collected in young adulthood (P56, $n = 11$–12/group) and analyzed for DA activity. Observations of control and defeat subjects following daily infusions of either vehicle, quinpirole, or amisulpride did not reveal any adverse behavioral effects of drug administration.

Data analysis

All analyses were performed using SigmaStat v.3.5, with α set at 0.05. Separate two-way ANOVA were used to analyze effects on DA turnover in the mPFC of age of social defeat, age sampled, or the effects of D_2 receptor ligands. Significant effects of age were followed by separate one-way ANOVA. Student–Newman–Keul's (SNK) *post hoc* tests were performed when interactions were significant.

For microdialysis data, post-stress DA peak heights were expressed as a percentage of the mean DA peak heights from three baseline samples. DA levels were analyzed with respect to time and treatment group using a two-way ANOVA with one repeated measure (time). Significant effects of treatment at a given time-point were analyzed by SNK multiple comparison tests. Significant effects of time were followed by a one-way ANOVA with one repeated measure across time for each given treatment. Significant time-points were then identified by Holm–Sidak *post hoc* tests for multiple comparisons, with the sample collected immediately before the stress serving as the control sample.

Results

Experiment 1: testing if social defeat effects on mPFC DA activity are specific to adolescent stress

Repeated social defeat of male rats during mid-adolescence (P35–39) produced different outcomes on mPFC DA turnover when compared with social defeat occurring in adulthood (P70–74), as evidenced by a significant interaction between stress and age ($F_{1,\ 35} = 35.935$; $P < 0.001$). Rats defeated in adolescence showed decreased mPFC DA turnover when

measured 17 days later (P56) compared with age-matched controls (SNK; $P<0.001$; Fig. 1). In contrast, rats defeated in adulthood had increased mPFC DA turnover compared with their age-matched controls 17 days following the last defeat episode (P91, SNK; $P=0.008$; Fig. 1). As a consequence, rats defeated in adulthood had increased mPFC DA turnover as compared with rats defeated in adolescence (SNK; $P=0.004$; Fig. 1). Interestingly, control rats for the adult defeat group sampled at P91 showed reduced mPFC DA turnover when compared with control rats for the adolescent defeat group sampled at P56 (SNK; $P<0.001$; Fig. 1).

Experiment 2: the effects of social defeat on DA release in the mPFC during adolescence

The microdialysis probes sampled from the cingulate, prelimbic and infralimbic subregions of the mPFC (3.7 to 2.8 mm anterior to bregma; Paxinos and Watson 1997) and were similarly distributed in rats comprising control, social control, and defeat experiments (Fig. 2a). Exposure to a resident rat within the social defeat context only increased extracellular DA in the mPFC of adolescent rats that had prior experience with social defeat (Fig. 2b). There were main effects of stress ($F_{2,\ 16}=5.706$; $P=0.013$), time ($F_{10,159}=3.741$; $P<0.001$), and a significant interaction between stress and time ($F_{20,\ 159}=2.380$; $P=0.002$) on mPFC DA release. One-way ANOVA revealed an effect over time for defeated rats ($F_{10,\ 59}=4.531$; $P<0.001$), where mPFC DA levels were increased at 20 and 40 min following the onset of the exposure to the resident rat (Holm–Sidak; $P<0.001$ and $P=0.002$, respectively; Fig. 2b). DA levels did not significantly differ over time for either control or social control groups (Fig. 2b). Comparison of all three groups at each time point sampled showed that adolescent rats with prior social defeat experience had greater mPFC DA levels than both control and social control rats at 20–60 min following the onset of the stress (SNK; P value range of 0.001–0.013), while mPFC DA levels did not differ between controls and social controls at any time point (Fig. 2b).

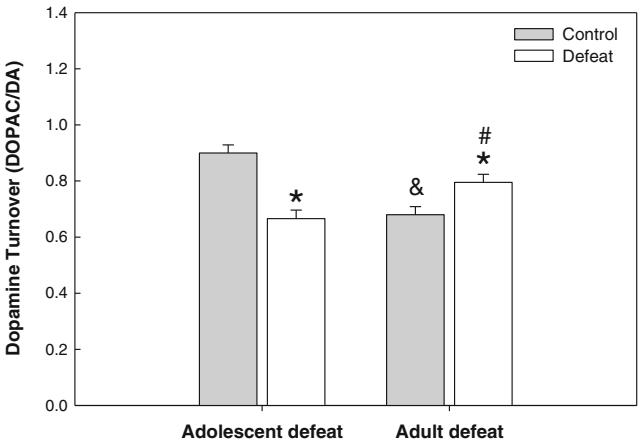

Fig. 1 DA activity (as a ratio of DOPAC/DA) in the mPFC of rats defeated in adolescence (P35–39) or adulthood (P70–74), as sampled 17 days following the last defeat episode (at P56 or P91). Data represent mean±SEM. *$P<0.05$, significantly different from age-matched controls; $^{\#}P<0.05$, significantly different from adolescent defeat group; $^{\&}P<0.05$, significantly different from adolescent control group. $N=10$ per group

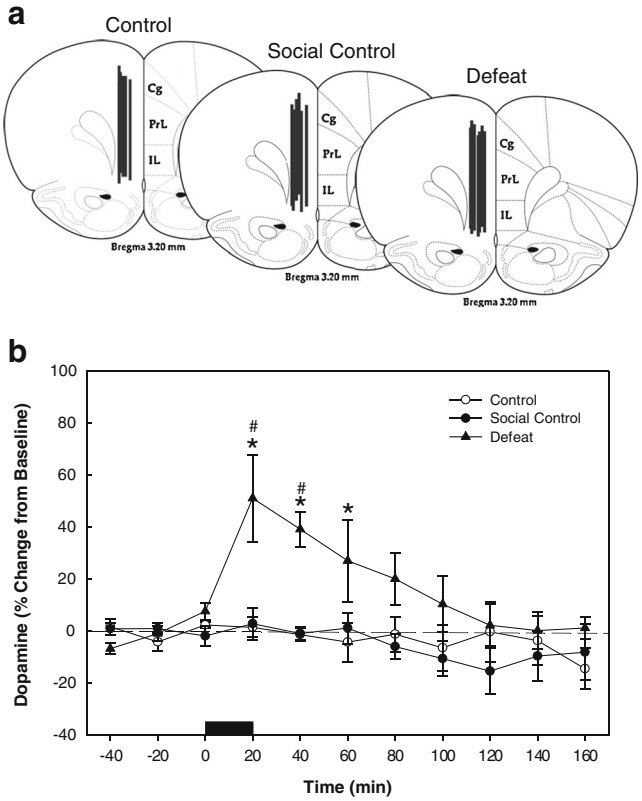

Fig. 2 **a** Representative schematic diagrams of microdialysis probe placements in the mPFC (AP, +3.2 mm; Paxinos and Watson 1997). *Cg* cingulate, *PrL* prelimbic, *IL* infralimbic. **b** Effects of exposure to social threat from a resident male rat (or empty resident home cage for controls) on mPFC extracellular DA levels in adolescent (P38) rats either previously exposed to social defeat or to a resident male without defeat (social control) or to an empty cage (control). *Horizontal bar* time exposed to resident and/or cage. Data represent mean±SEM. *$P<0.05$, significantly different from both control groups; $^{\#}P<0.05$, significantly different across time as compared with pre-stress levels. $N=6$–7 per group

Experiment 3: timeline for the reduction of mPFC DA activity following adolescent social defeat

The effects of adolescent social defeat (P35–39) on mPFC DA activity differed depending on the age at sampling, with a significant interaction between stress and age sampled ($F_{2,\ 44}=18.179$; $P<0.001$; Fig. 3). Socially defeated rats had increased mPFC DA turnover at P40 (SNK; $P<0.001$) and P49 (SNK; $P=0.021$), and reduced mPFC DA turnover at P56 (SNK $P=0.002$) compared with age-matched controls (Fig. 3). There was also a main effect of sampling age on mPFC DA turnover ($F_{2,\ 44}=9.907$; $P<0.001$; Fig. 3), which

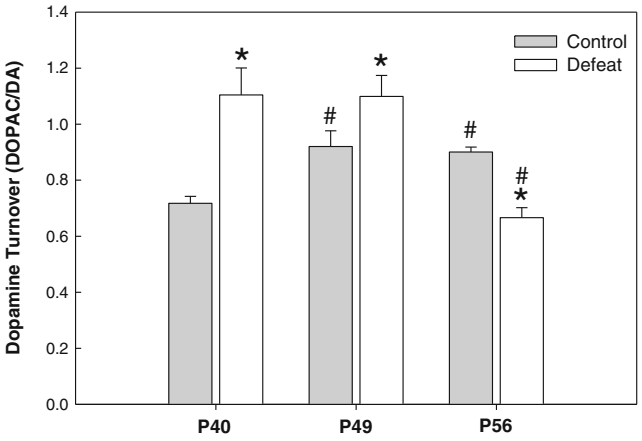

Fig. 3 DA activity (as a ratio of DOPAC/DA) in the mPFC of rats defeated in adolescence (P35–39), sampled at P40, P49, or P56. Data represent mean±SEM. *$P<0.05$, significantly different from age-matched controls; #$P<0.05$, significantly different from P40 and P49 for defeated rats or from P40 only for control group. $N=7$–10 per group

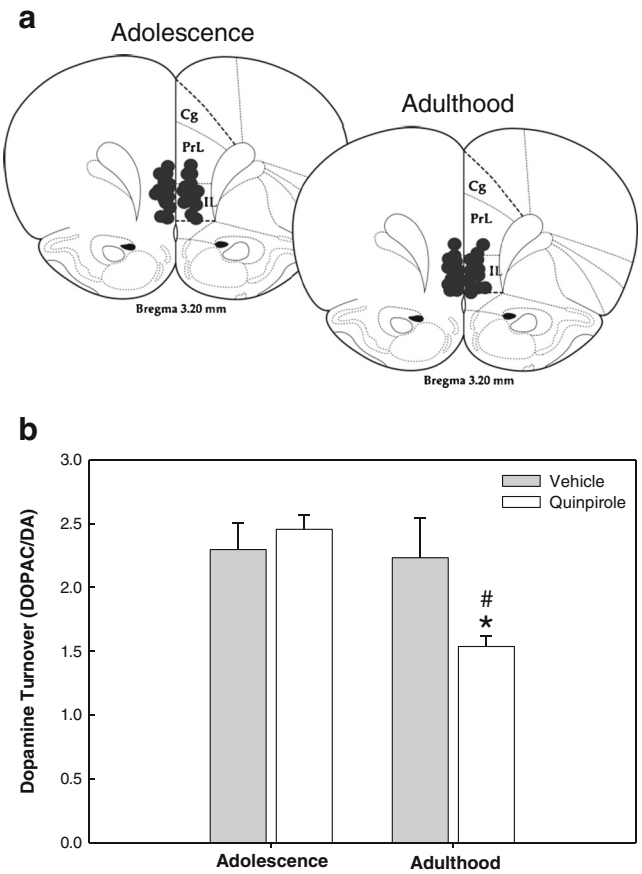

Fig. 4 **a** Representative schematic diagrams of bilateral quinpirole or vehicle infusion placements in the mPFC (AP, +3.2 mm; Paxinos and Watson 1997). *Cg* cingulate, *PrL* prelimbic, *IL* infralimbic. **b** DA activity (as a ratio of DOPAC/DA) in the mPFC of non-defeated rats infused daily with quinpirole or vehicle during adolescence (P35–39). Rats were sampled in adolescence (P40) or early adulthood (P56). Data represent mean±SEM. *$P<0.05$, significantly different from age-matched vehicle group; #$P<0.05$, significantly different from quinpirole group sampled in adolescence. $N=8$–9 per group

was apparent in both defeated ($F_{2,22}=14.013$; $P<0.001$) and control ($F_{2,24}=8.609$; $P=0.002$; Fig. 3) rats. Rats defeated in adolescence had lower mPFC DA turnover at P56 as compared with P40 and P49 (SNK $P<0.001$; Fig. 3). In contrast, mPFC DA turnover in control rats was higher at P49 and P56 when compared with P40 (SNK; $P=0.002$; Fig. 3).

Experiment 4: the role of D_2 receptors in adolescent defeat-induced decreases in mPFC DA activity

The drug infusion cannulae were located in the ventral prelimbic and infralimbic subregions of the mPFC (AP, +3.7 to 2.8 mm; Paxinos and Watson 1997), ensuring infusion into the ventral mPFC (Figs. 4a and 5a). Placements were similarly distributed among treatment groups for both D_2 receptor agonist and antagonist experiments (Figs. 4a and 5a).

Infusions of the D_2 receptor agonist quinpirole into the mPFC during adolescence (P35–39) had a differential effect on mPFC DA turnover depending on age of sampling (Fig. 4b), with a significant interaction between drug treatment and sampling age ($F_{1,32}=4.822$; $P=0.035$). There was no significant difference in mPFC DA turnover between vehicle- and quinpirole-treated rats when sampled during adolescence (P40). However, mPFC DA turnover was lower in quinpirole-treated rats sampled in young adulthood (P56; SNK; $P=0.020$; Fig. 4b). In addition, DA turnover was not significantly different in vehicle-infused adolescent and adult rats, but mPFC DA turnover was reduced in quinpirole-infused rats sampled in adulthood compared with those sampled in adolescence (SNK; $P=0.002$; Fig. 4b).

Intra-mPFC infusions of the D_2 receptor antagonist amisulpride into the mPFC prior to each defeat episode in adolescence (P35–39) prevented defeat-induced reductions in mPFC DA turnover in early adulthood (Fig. 5b). There was a significant interaction between stress and drug ($F_{1,42}=6.526$; $P=0.014$) for mPFC DA turnover. Defeated rats infused with vehicle exhibited the expected decrease in mPFC DA turnover as compared with vehicle-infused control rats (SNK; $P=0.036$; Fig. 5b). This effect was prevented by amisulpride pretreatment of the mPFC (defeat vehicle vs. defeat amisulpride SNK; $P=0.028$; Fig. 5b). Control rats infused with amisulpride during adolescence did not show significantly altered mPFC DA turnover as young adults when compared with vehicle-infused control rats (Fig. 5b).

Discussion

The results of this study compliment earlier findings demonstrating the sensitivity of the mPFC DA system to stressful situations, particularly those comprising agonistic social interactions (Tidey and Miczek 1996; Watt et al. 2009). In contrast

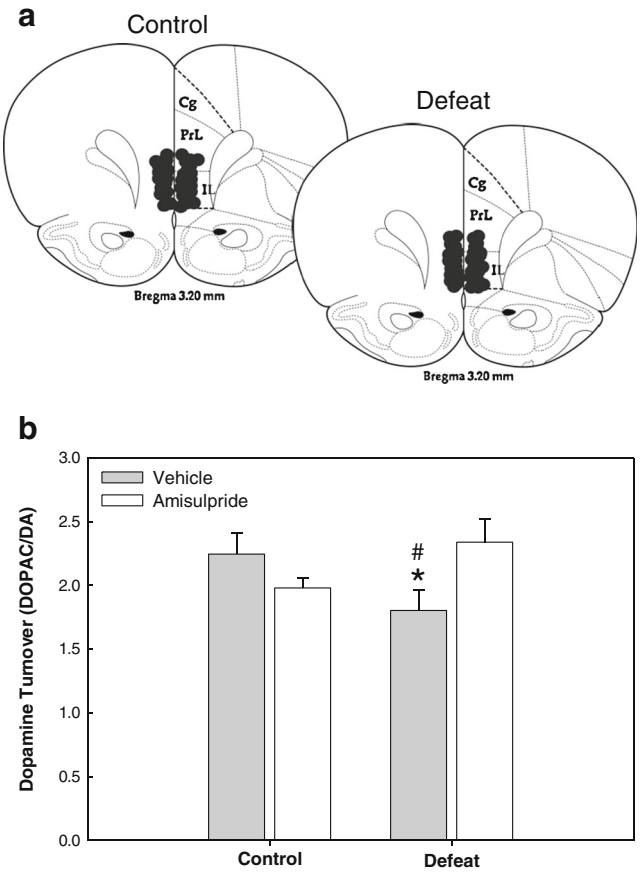

Fig. 5 **a** Representative schematic diagrams of bilateral amisulpride or vehicle infusion placements in the mPFC (AP, +3.2 mm; Paxinos and Watson 1997). *Cg* cingulate, *PrL* prelimbic, *IL* infralimbic. **b** DA activity (as a ratio of DOPAC/DA) in the mPFC of rats infused with amisulpride or vehicle prior to adolescent social defeat (P35–39). Rats were sampled in early adulthood (P56). Data represent mean±SEM. $^{*}P<0.05$, significantly different from defeat amisulpride group; $^{\#}P<0.05$, significantly different from vehicle control group. $N=11–12$ per group

to the decreased mPFC DA activity observed following adolescent social defeat, rats defeated in adulthood displayed increased mPFC DA activity 17 days beyond the last defeat episode, which may result from sustained activity of DA neurons in the VTA in the absence of further defeat stress (Nikulina et al. 2004). Therefore, while social defeat has persistent effects on mPFC DA activity regardless of when the stressor is experienced, the actual outcome is opposite in nature. The current findings thus support the hypothesis that the developing adolescent PFC DA system responds differently from the mature adult brain to stress-induced insult.

Decreased or increased mPFC DA activity as a consequence of social stress during adolescence or adulthood, respectively, could have similar outcomes on some behavioral measures. For example, sensitization to psychostimulants and salience of psychostimulant-associated cues can be enhanced by either decreased or excessive PFC DA activity (Beyer and Steketee 1999; Everitt and Wolf 2002; Ventura et al. 2004; Kalivas et al. 2005; Burke et al. 2011, 2013), and both adolescent and adult social defeat increase behavioral sensitivity to psychostimulants (Nikulina et al. 2004; Covington and Miczek 2005; Miczek et al. 2008, 2011; Burke et al. 2011, 2013). However, mPFC DA hypofunction due to social stress experienced in adolescence may also result in additional negative behavioral outcomes that would not occur as a result of adult social stress exposure. For instance, marked impairments in attentional set shifting and delay discounting tasks are associated with PFC DA hypofunction, with task performance improved by drugs that preferentially increase PFC DA transmission (Berridge et al. 2006; Floresco and Magyar 2006). Deficits in fear extinction are also observed when mPFC DA is depleted (Morrow et al. 1999; Fernandez Espejo 2003; Pezze and Feldon 2004). Thus, PFC DA hypofunction following adolescent defeat may potentially result in behavioral impairments unique from those caused by defeat in adulthood. This could be clarified by directly comparing the effects of adolescent and adult social defeat on these behaviors.

While adolescent social defeat resulted in decreased DA activity when measured in early adulthood, these rats actually showed increased mPFC DA release during exposure to threat of further defeat in adolescence, directly comparable to that seen in socially threatened adult rats (Tidey and Miczek 1996). Thus, social defeat stress appears to have similar acute effects on mPFC DA release whether experienced in adolescence or adulthood. Release of DA in the mPFC during stressful situations such as social threat is thought to enhance attentional focus and facilitate adaptive coping (Tidey and Miczek 1996; Pani et al. 2000). However, we hypothesize that sustained mPFC DA release during repeated adolescent defeat results in prolonged over-activation of presynaptic D_2 autoreceptors to ultimately cause long-term reductions in mPFC DA activity (Fig. 6). This is supported by the finding that repeated pharmacological activation of mPFC D_2 receptors in nondefeated adolescent rats, using a drug concentration shown to have preferential effects on D_2 autoreceptors (Doherty and Gratton 1999), decreased mPFC DA activity in early adulthood (Fig. 4b) to the same degree as adolescent defeat. By contrast, blockade of mPFC D_2 receptors during adolescent defeat at a drug concentration known to antagonize autoreceptor-mediated function (Barik and de Beaurepaire 1996) successfully prevented subsequent reductions in DA activity (Fig. 5b). Combined, these results suggest a D_2 autoreceptor mechanism underlies decreases in mPFC DA activity caused by adolescent defeat.

These findings raise a number of possibilities as to how D_2 autoreceptors activated in adolescence would contribute to deficits in adult mPFC DA function. First, increased DA release/activity during and after the stress experience may increase autoreceptor expression and function, resulting in greater inhibition of DA activity in the absence of stress (Fig. 6). This is supported by findings that physical and social stress causes delayed upregulation of D_2 receptors in terminal

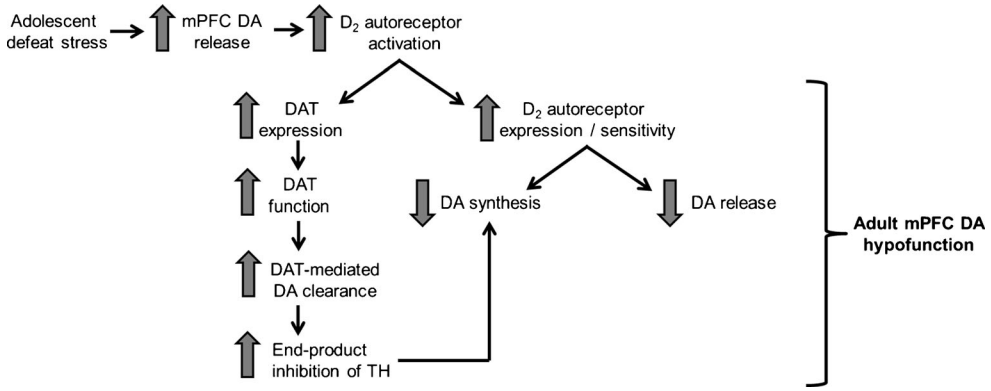

Fig. 6 Hypothesized progression of events by which adolescent defeat results in adult mPFC DA hypofunction. Repeated defeat stress elicits mPFC DA release and increases DA activity both during and after stress exposure, which leads to prolonged activation of D_2 autoreceptors from mid to late adolescence. This may increase D_2 autoreceptor expression and/or sensitivity, which would enhance inhibition of DA release and may also indirectly decrease DA synthesis. Alternatively, adolescent social defeat may interfere with the developmental transformation of mPFC D_2 autoreceptor function, resulting in D_2 autoreceptors that retain the ability to directly inhibit tyrosine hydroxylase (TH) into early adulthood. Finally, activation of D_2 autoreceptors could promote increased DAT expression and function, increasing DA clearance and uptake and lowering extracellular DA availability. Such an effect would increase intra-terminal DA concentrations to enhance end-product inhibition of TH and contribute to a decrease in DA synthesis. The combination of reduced DA release, increased DA clearance, and decreased DA synthesis would then promote the hypofunctional mPFC DA system seen in early adulthood. See text for relevant citations

DA fields in adult rats (MacLennan et al. 1989; Lucas et al. 2004). However, no change to adult mPFC D_2 receptor expression was observed following adolescent social defeat (Burke et al. 2011), although that study did not differentiate presynaptic and postsynaptic D_2 receptors. In subcortical regions, D_2 autoreceptors exert a direct inhibitory effect on tyrosine hydroxlase activity to reduce DA synthesis (Wolf et al. 1986), but this synthesis-modulating effect is only observed until age P40 in the rat PFC (Andersen et al. 1997). Therefore, a second possibility is that adolescent social defeat may alter developmental trajectories to result in persistence of D_2 autoreceptors with the capacity to decrease mPFC DA synthesis into early adulthood. Finally, mPFC D_2 autoreceptor activation during adolescent defeat may trigger other mechanisms that contribute to decreased DA activity. For instance, activation of D_2 autoreceptors increases gene transcription mediated by the orphan nuclear receptor Nurr1 (Kim et al. 2006), and Nurr1 activation increases transcription of the DA transporter (DAT) gene (Sacchetti et al. 2001). Defeat-induced activation of mPFC D_2 auotreceptors during adolescence may therefore increase Nurr1 activity to increase DAT expression in early adulthood, which could enhance DA clearance and subsequently reduce DA activity (Fig. 6). In support of this, we have shown that rats defeated in adolescence exhibit increased mPFC DAT expression as young adults (Novick et al. 2011), which preliminary data suggest results in greater DA clearance in adult rats defeated in adolescence (Novick et al. 2012). However, the possible contribution of D_2 receptors located on non-DAergic postsynaptic sites cannot be ruled out. While activation of D_2 receptors located on fast-spiking GABAergic mPFC interneurons has a paradoxical excitatory effect in the adult rat brain, these D_2 receptors are weakly inhibitory in adolescence (O'Donnell 2010). Thus, defeat-induced extracellular DA in the mPFC of adolescent rats may have a disinhibitory effect on further DA release, with repeated activation leading to down-regulation of these D_2 receptors on GABAergic cells, or to hastening their developmental transition to becoming excitatory. In this case, GABAergic interneurons may begin to exert an inhibitory influence on mPFC DA release and contribute to the reductions in DA activity following adolescent defeat. This possibility requires further study.

An interesting observation from control rats was an increase in mPFC DA turnover from mid-adolescence (P40) to early adulthood (P56), which matches the developmental increase in PFC DA fiber density over this time (Kalsbeek et al. 1988). It should be noted that control rats undergoing surgery and vehicle infusions prior to sampling did not show this developmental pattern (Fig. 4), which may reflect that such experimental manipulations artificially increase mPFC DA activity in adolescent rats. Interestingly, controls sampled in later adulthood (P91) exhibited lower mPFC DA activity than those sampled in early adulthood (P56). This increase and subsequent decline in mPFC DA activity from mid-adolescence to later adulthood resembles the pattern seen in expression of mPFC DA receptors across a similar developmental span (Andersen et al. 2000). Thus, developmental changes in mPFC DA activity from mid-adolescence to mid-adulthood are likely related to mPFC DA fiber density and receptor expression. It is also possible that adolescent social stress accelerates the normal developmental trajectory of mPFC DA activity as young adult rats previously exposed to adolescent social defeat show levels of mPFC DA activity more reminiscent of those seen in later adulthood.

Exposure to stress during childhood and adolescence has detrimental effects on cognitive ability and behavioral control in later life (Majer et al. 2010; McCormick et al. 2012; Sandstrom and Hart 2005; Sterlemann et al. 2010). These deficits appear to relate to the vulnerability of the developing PFC to stress-induced disruption, particularly by social stress (Leussis et al. 2008; Watt et al. 2009; Coppens et al. 2011; Novick et al. 2011). While adolescent and adult defeat exposure both alter mPFC DA activity, the DA hypofunction caused by repeated social defeat is unique to adolescence. Interestingly, many psychological disorders associated with experience of negative adolescent social experience are characterized by later deficits in many aspects of executive function (Hawker and Boulton 2000; Kaltiala-Heino et al. 2000; Brunstein Klomek et al. 2007; Brown 2008; Holmberg and Hjern 2008; Andersen and Teicher 2008, 2009), implying mPFC DA hypofunction. Our results suggest that stress-induced activation of mPFC D_2 receptors during adolescence promotes subsequent reductions in DA activity, and may represent a potential target for treating disorders associated with adolescent stress in which executive function is compromised.

Acknowledgments This work was supported by NSF IOS 1257679 (MJW), NIDA RO1 DA019921 (GLF), Joseph F. and Martha P. Nelson grants (AMN, KJR, and MJW), and NIH P20 RR015567, which is designated a Center of Biomedical Research Excellence (COBRE). The authors thank Kathryn Oliver, Eric Haaland, Mark Mingo, James Hassell, and Shaydie Engel for valuable technical assistance.

Conflict of interest The authors declare no conflict of interest.

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