Prefrontal cortical D<sub>1</sub> dopamine receptors modulate subcortical D<sub>2</sub> dopamine receptor-mediated stress responsiveness

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Abstract

Increased responsiveness to stress plays an important role in the manifestation of schizophrenia symptoms. Evidence indicates that the prefrontal cortex (PFC), and dopamine neurotransmission in the PFC in particular, is involved in the modulation of stress responsiveness. Decreased dopaminergic activity and loss of dopamine fibres have been reported in PFC in schizophrenia patients. Consequently, it was hypothesized that depletion of dopamine in PFC may facilitate increased stress responsiveness. Adult Sprague–Dawley rats received injections of 6-hydroxydopamine or saline bilaterally into the medial PFC (mPFC) following desipramine pretreatment to selectively deplete dopaminergic fibres. Following a 3-wk recovery period, the lesioned and control rats received injections of a D<sub>1</sub> or D<sub>2</sub> dopamine receptor agonist or vehicle into the mPFC and were immediately subjected to forced swimming as a stressor. Results showed that frequency of locomotion and rearing, behavioural measures indicative of increased dopaminergic activity in the nucleus accumbens (NAc), were significantly increased following stress in prefrontal cortical dopamine-depleted rats. This effect was significantly ameliorated by infusions of a D<sub>1</sub> dopamine receptor agonist directly into the mPFC in a dose-dependent manner but not by infusion of a D<sub>2</sub> dopamine receptor agonist. In addition, stress-induced behavioural changes in prefrontal cortical dopamine-depleted rats were significantly reduced following selective discrete infusions of a D<sub>2</sub> dopamine receptor antagonist into the NAc shell. The results suggest that dopaminergic transmission via D<sub>1</sub> receptors in the mPFC modulates D<sub>2</sub> dopamine receptor-mediated stress responsiveness in the NAc, a feature that may be disrupted in schizophrenia patients.

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Introduction

Exposure to psychosocial and biological stressors can exacerbate the symptoms of schizophrenia as well as trigger relapse (Norman & Malla, 1993a, b; Walker et al. 2007; Walker & Diforio, 1997). Stress activates dopaminergic neurons of the ventral tegmental area (VTA) resulting in the prompt release of dopamine from synaptic terminals located in the nucleus accumbens (NAc), amygdala and the prefrontal cortex (PFC) (Deutch & Roth, 1990; Doherty & Gratton, 1992, 1996; Finlay & Zigmond, 1997; McKittrick & Abercrombie, 2007; Pacchioni et al. 2007; Pascucci et al. 2007; Rosenkranz & Grace, 2002; Sullivan & Gratton, 1998; Thierry et al. 1976). Excessive efflux of dopamine in the NAc is thought to be associated with certain positive symptoms of schizophrenia (Carlsson & Lindqvist, 1963; Gray, 1995). However, the mechanisms controlling dopamine efflux in the NAc are not clear although the PFC is thought to be involved (Goto et al. 2007; Weinberger et al. 1988).

Evidence suggests that schizophrenia is associated with compromised prefrontal cortical dopamine neurotransmission (Davis et al. 1991; Franzen & Ingvar, 1975; Ingvar & Franzen, 1974; Weinberger, 1987, 1988). Schizophrenia patients show poor performance in tasks involving working memory and this is associated with reduced levels of homovanillic acid (HVA) in their cerebrospinal fluid (Weinberger, 1988;
Weinberger et al. 1988). HVA is a metabolite of dopamine and cerebrospinal fluid levels are closely correlated with dopaminergic activity in the PFC (Heritch, 1990; Kahn et al. 1994; Ribeyre et al. 1994). In addition, post-mortem studies have identified a loss of neurons thought to be dopaminergic in the VTA of brains from schizophrenia patients (Bogerts et al. 1983) and an average 35% loss of dopamine fibres in the deep layers of the PFC (Akil et al. 1999). They attributed the difference to the decreased re-uptake of dopamine through noradrenergic fibres, stress-induced dopamine efflux in animals with lesions involving noradrenergic fibres in animals with lesions involving noradrenergic fibres, stress-induced dopamine efflux in animals with larger lesions significantly decreased in the PFC compared to control rats (Venator et al. 1999). In-vivo imaging studies have also identified a decreased density of D2-like receptors in the PFC in drug-naïve schizophrenia patients (Okubo et al. 1997). Moreover, administration of D2 receptor agonists leads to improved activation of the PFC as measured with PET imaging, and improved performance on working-memory tasks (Abi-Dargham & Moore, 2003; Daniel et al. 1989, 1991; Dolan et al. 1995; Fletcher et al. 1996; Graud et al. 1987; Mu et al. 2007).

In rodents, bilateral depletion of dopamine in the medial PFC (mPFC) following local infusions of 6-hydroxydopamine (6-OHDA) was reported to augment spontaneous and amphetamine-induced locomotion, measures indicating increased dopaminergic activity in the NAc (Carter & Pycock, 1980; Pycock et al. 1980a,b; for review, see Tsentschke, 2001). Similar lesions were also found to increase stress-induced dopaminergic activity in the NAc (Deutch et al. 1990). However, a number of other studies have failed to find similar changes (Banks & Gratton, 1995; Clarke et al. 1988; Joyce et al. 1983; King & Finlay, 1995). Reasons for the discrepancies are not clear. Interestingly, Venator et al. (1999) described a 6-OHDA lesioning method that depleted ~63% of dopaminergic fibres in the PFC without affecting noradrenergic fibres. In these rats, stress-induced dopamine efflux was significantly decreased in the PFC compared to control animals. However, in animals with larger lesions resulting in the loss of both dopaminergic and noradrenergic fibres, stress-induced dopamine efflux in the PFC did not differ from that of control rats (Venator et al. 1999). They attributed the difference to the decreased re-uptake of dopamine through noradrenergic fibres in animals with lesions involving noradrenergic axons of the PFC (Venator et al. 1999). Therefore, some of the discrepancies seen in the above studies might be due to the extent of noradrenergic fibre loss following 6-OHDA-induced dopaminergic lesions. In the present study, regulation of dopaminergic activity in the NAc by dopaminergic neurotransmission in the mPFC following exposure to stress was investigated using an animal model with specific depletion of dopaminergic fibres, but not noradrenergic fibres, in the PFC (King & Finlay, 1995).

**Methods**

**Animals**

Male Sprague–Dawley rats aged 14–16 wk (Charles River, Canada) were used. Rats were housed in pairs in a controlled environment with constant temperature and humidity, a 12-h light/dark cycle (lights on 07:00 hours) and were provided with food and water ad libitum. All procedures were approved by the Institutional Animal Care Committee and are in compliance with the Canadian and National Institute of Health Guides for Care and Use of Laboratory Animals. All efforts were made to minimize pain and suffering.

**Surgical procedure**

The procedure described by King & Finlay (1995) was strictly followed to specifically deplete dopamine in the mPFC. Briefly, rats were pretreated with an intraperitoneal (i.p.) injection of desipramine hydrochloride (Sigma-Aldrich Canada Ltd, Canada; 25 mg/kg free base) to block the uptake of 6-OHDA into noradrenergic terminals (Gerfen & Clavier, 1981). After 20 min, these rats were anaesthetized with sodium pentobarbital (MTC Pharmaceuticals, Canada; 65 mg/kg i.p.) and placed on a Kopf stereotaxic frame. A fresh solution containing 6-OHDA (Sigma-Aldrich; 1 μg 6-OHDA in 2 μl of vehicle consisting of 0.05% ascorbic acid) was infused stereotaxically into the mPFC (Fig. 1c; AP +3.25 mm, ML ±0.7 mm from bregma, and DV −3.2 mm from dura; Paxinos & Watson, 1997). In total, 111 rats received 6-OHDA injections (lesioned) and an additional 111 animals received similar injections of vehicle alone (2 μl of 0.05% ascorbic acid per side, sham-operated). Two weeks after surgery, 63 sham-operated and 63 lesioned rats (randomly assigned) were re-anaesthetized, and 21-gauge guide cannulae were implanted bilaterally over the mPFC stereotaxically (same AP and ML coordinates as above but with DV coordinates of −2.2 mm; −1 mm above the target site allowing the inner cannulae to reach the target with minimum tissue damage) using dental acrylic, as outlined previously (Rajakumar et al. 1997). The remainder of the 48 lesioned and 48 sham-operated rats (randomly assigned) were anaesthetized and guide cannulae implanted bilaterally above the NAc shell (n=24 per group) or core (n=24 per group) using the stereotaxic coordinates of Paxinos & Watson (1997) (Fig. 1f; shell: AP +1.6 mm, ML ±0.8 mm from bregma, DV −7.0 mm from dura; core: AP +1.6 mm, ML ±1.5 mm from bregma, DV −7.0 mm from dura).
**Behavioural testing**

One week following cannulation, animals were placed in a Plexiglas testing chamber (50 $\times$ 50 $\times$ 30 cm$^3$) and left undisturbed for a minimum of 2 h to habituate to the environment. Baseline observations of locomotion and rearing were recorded for the final 5 min of the habituation period in order to ensure consistency among the rats. In the first series of studies, awake, behaving rats received bilateral infusions of one of three different doses of the full D$_1$ dopamine receptor agonist, SKF 81297 ($n=27$), the D$_2$ dopamine receptor agonist, quinpirole ($n=27$) or vehicle ($n=9$) directly into the mPFC. SKF 81297 (Sigma-Aldrich; 0.5 $\mu$m, 1 $\mu$m or 2 $\mu$m in sterile saline; 0.8 $\mu$l per side infused over 3 min; $n=9$ per group), (+)-quinpirole (Sigma-Aldrich; 5 $\mu$m, 10 $\mu$m or 20 $\mu$m in sterile saline; 0.8 $\mu$l per side infused over 3 min; $n=9$ per group) or vehicle (0.8 $\mu$l per side over 3 min; $n=9$) was infused using a Hamilton syringe and a microinfusion pump. Intermediate doses of SKF 81297 and quinpirole were selected based on studies by Bandyopadhyay et al. (2005). Immediately following drug infusion, the rats were exposed to an unavoidable stressor by means of forced swimming and their frequency of locomotion and rearing were visually monitored and recorded. Each animal was only exposed to a single swim stress to avoid learning-induced changes. Displacement of all four limbs was considered locomotion, and every occurrence from a stationary position was recorded.

Fig. 1. Photomicrographs showing tyrosine hydroxylase (TH) ($a$, $b$) and dopamine $\beta$-hydroxylase (DBH) ($c$, $d$) immunolabelling in the medial aspect of the PFC as shown in panel ($e$). Panels ($a$) and ($c$) are taken from sham-operated animals, while ($b$) and ($d$) are from 6-hydroxydopamine (6-OHDA)-lesioned rats. Note the plexus of TH-labelled fibres and varicosities in sham-operated animals [arrowhead in ($a$)] and are largely depleted in 6-OHDA-lesioned rats [arrowhead in ($b$)]. The fine varicosities of DBH labelling in sham-operated animals [arrows in ($c$)] appears similar to 6-OHDA-lesioned rats [arrows in ($d$)]. Panels ($g$) and ($h$) are photomicrographs showing TH-labelled fibres (arrows) in the shell of the nucleus accumbens as shown in ($f$), taken from sham-operated and 6-OHDA-lesioned rats, respectively. Note that the density of labelling between ($g$) and ($h$) appears comparable. Arrows in ($a$) and ($f$) show the infusion sites within the PFC, nucleus accumbens core ($c$) and nucleus accumbens shell. Bar ($a$–$d$), 140 $\mu$m; ($g$, $h$), 100 $\mu$m.
Similarly, standing on both hindlimbs with forelimbs raised was considered as rearing and recorded.

Forced swimming has been used previously in rodent studies to induce reproducible stress responses (Balkan et al. 2006; Bareggi et al. 1978; Nagy et al. 1983; Roth et al. 1982). It is important to note that the stress paradigm employed in the present study is different from the forced swimming test often used as a measure of depression in animal models involving two consecutive exposures to swim challenge (Cryan et al. 2005; Lucki, 1997; Forsolt et al. 1978, 1979). Each animal was placed for 5 min in a plastic cylinder (45 cm diameter × 120 cm height) half-filled with cold water (5–6 °C) in such a way that rats cannot reach the brim nor stand on the bottom. Upon removal from the water, the rats were quickly dried in paper towels and returned to the testing chamber within 2 min. The frequency of locomotion and rearing were visually monitored and recorded during 5-min blocks at 5–10 min, 20–25 min, 45–50 min of cessation of swim stress by an observer who was blind to the type of lesion.

Stress- and amphetamine-induced rearing and locomotion may involve dopaminergic activity in the NAc, striatum, PFC and/or the hippocampus (Mogenson et al. 1993; Whishaw & Dunnett, 1985). It is well known that NAc shell and core regions differ in neurochemical characteristics, connections and function (Deutch & Cameron, 1992; Jongen-Relo et al. 1993; Kalivas & Duffy, 1995; Zahm, 1992). Although stress and amphetamine cause dopamine efflux into the shell and core regions, the magnitude of dopamine release is much greater and long lasting within the core in normal animals (McKittrick & Abercrombie, 2007; Pacchioni et al. 2007). Depletion of dopamine in the PFC has been shown to differentially affect dopamine efflux within the shell and core of the NAc (King et al. 1997). Consequently, we proposed to determine whether the stress-induced behavioural changes following prefrontal cortical dopamine depletion are mediated preferentially via the core, shell or both regions. Rats received direct infusions of a D1/D2 dopamine receptor antagonist raclopride (Sigma-Aldrich) or saline bilaterally into the NAc shell (n = 24) or core (n = 24). Rats were habituated to the test area and baseline locomotion and rearing frequencies were recorded during the last 5 min of habituation. Following habituation, raclopride or saline [1 µg or 4 µg raclopride in 0.5 µl saline or 0.5 µl saline alone per site delivered over 3 min according to Baldo et al. (2002) and Calaminus & Hauber (2007)] was infused into the NAc shell or core region (n = 8 per dose each group). Rats were then exposed to an unavoidable stressor by means of forced swimming, and their frequency of locomotion and rearing were recorded. Finally, randomly selected rats (n = 4 per group of PFC or NAc core or shell) were infused via the cannula with 1% rhodamine (same volumes as above) and perfusion-fixed in 30 min to determine the accuracy of injection and the extent of diffusion.

In order to verify whether lesioning itself has an effect on locomotor behaviour, a separate group of lesioned (n = 18) and sham-operated (n = 18) rats were implanted with indwelling cannulae bilaterally over the PFC. Rats were habituated to the test area, baseline measures were taken and then they were infused with saline into the PFC. One-half of the group was subjected to forced swim as above (n = 9 lesioned and n = 9 sham-operated rats), and the other half was handled and sham dried. Their locomotor and rearing behaviours were recorded.

**Western blotting and immunohistochemistry**

Specificity and extent of dopamine depletion in the PFC was verified using Western blotting and immunohistochemistry. The day after behavioural testing, a randomly selected group of animals (6-OHDA lesioned, n = 10; sham-operated, n = 10) were decapitated and the brains rapidly removed. The prefrontal area rostral to the fusion of the corpus callosum (cingulate and prelimbic area) and the NAc (shell and core together) were microdissected separately and total protein extracted as described previously (Rushlow et al. 2005). Western blotting was conducted as outlined (Rushlow et al. 2005) using well-characterized antibodies for tyrosine hydroxylase (TH; mouse monoclonal, Sigma-Aldrich) and dopamine β-hydroxylase (DBH; mouse monoclonal, Chemicon, USA). The remainder of the lesioned and a random group of sham-operated rats (n = 12) were injected with a lethal dose of sodium pentobarbital (105 mg/kg i.p.) and transcardially perfused with saline, followed by 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and stored in 24% buffered-sucrose solution at 4 °C until sectioned in a freezing microtome. Sections were processed for TH or DBH immunohistochemistry using an avidin-biotin complex system and DAB/H2O2 as described previously (Rajakumar et al. 1994).

**Data analysis**

For the behavioural analysis, the data-points are presented as mean ± S.E.M. Statistical analysis was conducted using a one-way ANOVA followed by Tukey’s post-hoc test, and each time block was analysed
mPFC, TH immunolabelling revealed a fibre plexus coeruleus (respectively) in sham-operated rats vs immunolabelling in the ventral midbrain and locus striatus. Both TH and DBH antibodies provided characteristic fibres in the nucleus accumbens (Fig. 1c) whereas DBH labelling revealed thin fibres and fine varicosities (Fig. 1a). Relative to the sham-operated rats, the density of the thick fibres and varicosities labelled with TH was markedly reduced following 6-OHDA lesions (Fig. 1a, b). Both the fibre density and the spatial pattern of DBH labelling appeared similar between sham-operated and 6-OHDA-lesioned rats (Fig. 1c, d). TH immunohistochemistry showed comparable density of immunoreactive fibres in the NAc of both 6-OHDA-lesioned and sham-operated animals (Table 1, Fig. 1g, h).

Western blots generated using protein isolated from the mPFC of 6-OHDA-lesioned and sham-operated rats revealed a significant decrease in TH protein levels following lesioning but no change in DBH protein levels confirming the specific nature of the lesion (Fig. 2a). Western blotting of protein from the NAc punches revealed similar levels of TH and DBH between lesioned and sham animals (Fig. 2b). Both the TH and DBH antibodies yielded bands of the appropriate molecular weight. All lesioned rats showed comparable depletion of dopaminergic fibres bilaterally as revealed by immunohistochemistry or Western blotting, and consequently, they all were included in the behavioural analysis.

**Table 1. Optical density of tyrosine hydroxylase (TH)-labelled fibres in the nucleus accumbens**

<table>
<thead>
<tr>
<th></th>
<th>Shell</th>
<th>Core</th>
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<tr>
<td>6-hydroxydopamine (6-OHDA)</td>
<td>107.407 ± 11.208</td>
<td>126.472 ± 24.362</td>
</tr>
<tr>
<td>Sham</td>
<td>100.541 ± 18.727</td>
<td>121.716 ± 19.418</td>
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Optical density of TH immunoreactive fibres (in arbitrary units) in sections through the nucleus accumbens shell or core in 6-OHDA-lesioned or sham-operated control rats (n = 12 per group, mean ± s.e.m.).

For the Western blot data, densitometry values for TH and DBH proteins were corrected for background and protein loading using α-tubulin and the resultant values compared using a Student’s t test. Immunohistochemical staining intensity was measured with Image-J software (NIH, USA) and compared to corresponding control brains using a Student’s t test. Alpha was set at 0.05 for all comparisons.

**Results**

**Verification of lesion**

Both TH and DBH antibodies provided characteristic immunolabelling in the ventral midbrain and locus coeruleus (respectively) in sham-operated rats consistent with that reported in the literature. In the mPFC, TH immunolabelling revealed a fibre plexus consisting of thick fibres and coarse varicosities (Fig. 1a) whereas DBH labelling revealed thin fibres and fine varicosities (Fig. 1c). Relative to the sham-operated rats, the density of the thick fibres and varicosities labelled with TH was markedly reduced following 6-OHDA lesions (Fig. 1a, b). Both the fibre density and the spatial pattern of DBH labelling appeared similar between sham-operated and 6-OHDA-lesioned rats (Fig. 1c, d). TH immunohistochemistry showed comparable density of immunoreactive fibres in the NAc of both 6-OHDA-lesioned and sham-operated animals (Table 1, Fig. 1g, h).

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**Stress modulation via D1 dopaminergic receptors of the PFC**

In comparison to the sham-/vehicle-treated rats, the 6-OHDA-/vehicle-treated group showed a significant increase in the frequency of locomotion [5–10 min: F(1, 15) = 169.1, p < 0.05; 20–25 min: F(1, 15) = 133.3, p < 0.05; 40–45 min: F(1, 15) = 114.2, p < 0.05] and rearing [5–10 min: F(1, 15) = 165.9, p < 0.05; 20–25 min: F(1, 15) = 118.8, p < 0.05; 40–45 min: F(1, 15) = 100.4, p < 0.05; throughout the analysis, baseline values were treated as covariate] following exposure to an acute stressor at all three time blocks examined, suggesting that depletion of dopamine in the mPFC may increase stress responsiveness to acute environmental stressors (Fig. 3a–d). Prefrontal cortical infusions of SKF 81297, a full agonist on D1 dopamine receptors, at the lowest dose employed (0.5 μM) significantly suppressed the frequency of locomotion at 5–10 min and 20–25 min time-periods, and rearing at the 5–10 min time-period [locomotion: 5–10 min: F(1, 15) = 39.45, p < 0.05; 20–25 min: F(1, 15) = 132.15, p < 0.05; 40–45 min: F(1, 15) = 34.99, p = 0.895; rearing: 5–10 min: F(1, 15) = 13.6, p < 0.05; 20–25 min: F(1, 15) = 114.41, p = 0.065; 40–45 min: F(1, 15) = 33.07, p = 0.145] compared to vehicle infusions (Fig. 3a, b). Significant suppressions of locomotion and rearing were seen following infusions of 1 μM SKF 81297 at both 5–10 min and 20–25 min time blocks [locomotion: 5–10 min: F(1, 15) = 14.68, p < 0.05; 20–25 min: F(1, 15) = 93.33, p < 0.05; 40–45 min: F(1, 15) = 90.73, p = 0.315; rearing: 5–10 min: F(1, 15) = 5.74, p < 0.05; 20–25 min: F(1, 15) = 81.42, p < 0.05; 40–45 min: F(1, 15) = 77.14, p = 0.096]. Infusions of 2 μM SKF 81297 into the mPFC suppressed the locomotion or rearing at all three time blocks [locomotion: 5–10 min: F(1, 15) = 3.41, p < 0.05; 20–25 min: F(1, 15) = 23.21, p < 0.05; 40–45 min: F(1, 15) = 2.98, p < 0.05; rearing: 5–10 min: F(1, 15) = 1.02, p < 0.05; 20–25 min: F(1, 15) = 8.62, p < 0.05; 40–45 min: F(1, 15) = 3.15, p < 0.05]. Interestingly in sham-operated animals, at 5–10 min and 20–25 min time blocks, both behavioural measures were significantly suppressed by SKF 81297 in a dose-dependent manner in comparison to behaviours following infusions of vehicle [locomotion: 5–10 min: F(1, 15) = 2.71, p < 0.05; 20–25 min: F(1, 15) = 8.79, p < 0.05; rearing: 5–10 min: F(1, 15) = 1.61, p < 0.05; 20–25 min: F(1, 15) = 6.7, p < 0.05] (Fig. 3a, b). In contrast to SKF 81297,
prefrontal cortical infusions of (±)-quinpirole failed to alter stress-induced behaviours at all three time blocks for all three doses tested (F values are not given for clarity; Fig. 3c, d). Infusions of rhodamine via the cannulae showed that the rhodamine labelling was confined to the prelimbic area and the adjacent part of the anterior cingulate cortex. Finally, 6-OHDA-lesioned and sham-operated rats that were not exposed to forced swim stress did not differ in their behavioural measures at baseline and at any of the testing periods (Table 2; F values are omitted for clarity) indicating lesion alone does not significantly affect the locomotor behaviour.

**Stress-induced locomotion and rearing are mediated via D2 dopaminergic receptors in the NAc shell and core**

In order to verify whether the increased locomotion and rearing seen in prefrontal cortical dopamine-depleted rats following exposure to stressors was mediated by D2 dopamine receptors in the NAc, different groups of rats were directly infused with two different doses of the D2/3 dopamine receptor blocker raclopride into the NAc shell or core regions. Infusions of raclopride into the NAc shell significantly suppressed stress-induced increase in locomotion and rearing behaviours at 5–10 and 20–25 min time-points in a dose-dependent manner [1 µg dose: locomotion: 5–10 min: F(1, 13) = 201.38, p < 0.05; 20–25 min: F(1, 13) = 123.47, p < 0.05; rearing: 5–10 min: F(1, 13) = 81.05, p < 0.05; 20–25 min: F(1, 13) = 105.55, p < 0.05] (Fig. 4a, b). In addition, the higher dose of raclopride (4 µg) markedly reduced both behaviours throughout the testing period in sham-operated rats [locomotion: 5–10 min: F(1, 13) = 151.35, p < 0.05; 20–25 min: F(1, 13) = 74.95, p < 0.05; rearing: 5–10 min: F(1, 13) = 11.33, p < 0.05; 20–25 min: F(1, 13) = 3.51, p < 0.05; 40–45 min: F(1, 13) = 2.21, p < 0.05] (Fig. 4a, b).

Infusions of raclopride into the core region of the NAc showed significant suppression of stress-induced locomotion and rearing at 5–10 and 20–25 min time blocks in prefrontal cortical dopamine-depleted rats.
Prefrontal dopamine on stress response

following the higher dose (4 μg), but not with the low dose [4 μg dose, locomotion: 5–10 min: F(1, 13) = 74.15, p < 0.05; 20–25 min: F(1, 13) = 51.74, p < 0.05; 40–45 min: F(1, 13) = 6.84, p = 0.043; rearing: 5–10 min: F(1, 13) = 17.33, p < 0.05; 20–25 min: F(1, 13) = 6.31, p < 0.05; 40–45 min: F(1, 13) = 2.75, p = 0.085] (Fig. 4c, d). The frequencies of locomotion and rearing seen after the high dose of raclopride (4 μg) infusions were still significantly higher than those seen in sham-operated rats indicating the suppression of stress-induced behaviours were only partially suppressed by infusions of raclopride in to the core region [locomotion: 5–10 min: F(1, 13) = 36.75, p < 0.05; 20–25 min: F(1, 13) = 21.35, p < 0.05; and rearing: 5–10 min: F(1, 13) = 2.85, p < 0.05; 20–25 min: F(1, 13) = 1.95, p < 0.05] (Fig. 4c, d).

In addition, unlike infusions into the shell, raclopride in the core did not affect locomotion and rearing behaviours in sham-operated rats. Representative animals that received infusions of rhodamine either into the core or the shell revealed that the in-vivo spread was mainly confined to the core or the medial aspect of shell, respectively.

Discussion

The present results indicate that depletion of dopamine in the mPFC augments certain behavioural manifestations following exposure to acute stress. This effect is dependent on the suppressed D<sub>1</sub> dopamine receptor activity of the mPFC, and is mediated by D<sub>2</sub> dopamine receptor activity of the NAc shell and possibly by the core region. The present finding agrees
with the current view of pathophysiology underlying stress-induced exacerbation of positive symptoms in schizophrenia (Norman & Malla, 1993a, b; Walker et al., 2007).

The lesioning method employed in the present study strictly followed the procedure described by King & Finlay (1995). Previously, these investigators were able to demonstrate that selective lesioning of the mPFC with 6-OHDA results in ~80% depletion of dopamine levels but only a 12% depletion of noradrenaline in the mPFC (King & Finlay, 1995). In agreement with this finding, the present data showed a marked decrease in TH protein levels in the PFC, while DBH protein levels were not significantly affected. TH is found in both dopaminergic and noradrenergic fibres. The Western blotting results were supported by the immunohistochemical observations that showed a comparable density and spatial distribution of DBH immunoreactive fibre plexus in the mPFC of 6-OHDA-lesioned and sham-operated rats indicating sparing of noradrenergic fibres. Collectively, the results indicate that the lesioning methods employed in the present study preferentially deplete dopamine in the PFC without significantly affecting noradrenergic axons. As shown previously, specificity of the lesion is critical for interpretation of the results. Depletion of noradrenergic and dopaminergic fibres in the PFC produced comparable dopamine efflux in depleted and control animals following exposure to stress, while sparing noradrenergic fibres and depleting dopaminergic fibres in the PFC resulted in decreased prefrontal cortical dopamine efflux in lesioned rats compared to controls following exposure to stress (Venator et al. 1999). In addition, both dopamine and noradrenaline neurotransmission in the PFC has been shown to modulate subcortical dopaminergic activity (Darraç et al. 1998; McKittrick & Abercrombie, 2007; Nicnocaill & Gratton, 2007; Pascucci et al. 2007; Tassin, 1998; Ventura et al. 2003).

A comparable density of dopamine immunoreactive fibres and TH protein levels were seen in the NAc of 6-OHDA-lesioned and sham-operated animals. This suggests that lesioning did not affect dopaminergic fibres in the NAc and therefore the behavioural changes observed are unlikely to be due to post-synaptic dopamine receptor sensitization that often follows loss of striatal dopamine fibres (Cai et al. 2002). In addition, despite bilateral lesioning, food intake and body weights were comparable between 6-OHDA-lesioned and sham-operated rats throughout the experiment (data not shown) indicating that the lesion did not disrupt the subset of VTA dopaminergic neurons involved in feeding behaviour (Korotkova et al. 2003; Taber & Fibiger, 1997).

Prefrontal cortical modulation of stress-induced changes in dopamine neurotransmission has previously been described (Deutch & Roth, 1990; King et al. 1997; Rosin et al. 1992). Microinjections of D1 receptor antagonist SCH 23390 directly into the mPFC significantly increased the NAc stress response in normal rats indicating that stimulation of D1 dopamine

<table>
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<th>Group</th>
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<th>5–10 min</th>
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<tr>
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<td>3 ± 0.8</td>
<td>1 ± 0.5</td>
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<tr>
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<td>5 ± 3.1</td>
<td>4 ± 2.0</td>
<td>3 ± 1.7</td>
</tr>
<tr>
<td>6-OHDA/stress</td>
<td>2 ± 1.1</td>
<td>23 ± 4.8*</td>
<td>15 ± 2.5*</td>
<td>9 ± 2.1*</td>
</tr>
<tr>
<td>Frequency of rearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control/no stress</td>
<td>1 ± 0.7</td>
<td>2 ± 1.9</td>
<td>2 ± 1.5</td>
<td>1 ± 0.4</td>
</tr>
<tr>
<td>Control/stress</td>
<td>0</td>
<td>4 ± 1.4</td>
<td>2 ± 1.7</td>
<td>2 ± 0.8</td>
</tr>
<tr>
<td>6-OHDA/no stress</td>
<td>2 ± 1.0</td>
<td>4 ± 2.2</td>
<td>1 ± 0.8</td>
<td>2 ± 1.1</td>
</tr>
<tr>
<td>6-OHDA/stress</td>
<td>2 ± 1.4</td>
<td>12 ± 3.7*</td>
<td>8 ± 2.9*</td>
<td>5 ± 1.6*</td>
</tr>
</tbody>
</table>

6-OHDA, 6-hydroxydopamine.

Frequencies of locomotion and rearing recorded for 5-min time blocks during the baseline, 5–10 min, 20–25 min and 40–45 min following cessation of forced swim stress or sham stress in 6-OHDA-lesioned rats or sham-operated control animals (n = 9 per group, mean ± S.E.M.; * p < 0.05 compared to other three groups). Note that spontaneous behaviours (no-stress groups) were not significantly different between 6-OHDA-lesioned and sham-operated control rats.
receptors in the mPFC may dampen stress-induced dopaminergic activity in the NAc, and loss of this effect might increase subcortical dopaminergic activity following exposure to stress (Doherty & Gratton, 1996). In agreement with this hypothesis, microinfusions of the D$_1$ receptor agonist SKF 81297 significantly suppressed both behavioural measures in sham-operated animals, at 5–10 and 20–25 min time blocks in comparison to vehicle infusions (*p < 0.05). Asterisks are placed underneath in panel (b) to increase the clarity. Following the high dose of raclopride (4 μg), frequencies of locomotion and rearing at 5–10 and 20–25 min time blocks were significantly reduced (p < 0.05) and they were comparable to that seen in sham-operated rats with vehicle infusions. In addition, the high dose of raclopride significantly suppressed both locomotion and rearing at all time-points examined in sham-operated rats compared to vehicle infusions (‡p < 0.05). In contrast to this, infusions of raclopride into the core region in 6-OHDA-lesioned rats showed significant suppression of locomotion (c) and rearing (d) only after infusions of higher doses (*p < 0.05), and the frequencies were still significantly higher than seen in sham-operated animals (‡p < 0.05) (mean ± S.E.M., n = 8 per group; *p < 0.05).

Present results also indicate that certain stress effects mediated via the D$_2$ receptors of the mPFC are manifested through the activity of D$_2$ dopamine receptors of the NAc. Dopaminergic activity of the NAc is modulated by direct prefrontal cortical efferent projections to the NAc (Youngren et al. 1993) as well as by indirect projections via the VTA (Karreman & Moghaddam, 1996; Taber et al. 1995). According to Carr & Sesack (2000), prefrontal cortical efferent fibres contact dopaminergic and GABAergic neurons of the VTA. These dopaminergic neurons either project to the PFC or to the brainstem, but not directly to the NAc. The GABAergic neurons targeted by the PFC...
efferents either project to the NAc or may contact the dopaminergic neurons of the VTA that project to the NAc thereby modulating mesocortical dopaminergic activity (Carr & Sesack, 2000). In addition to the PFC–VTA projection, GABAergic medium spiny neurons of the NAc send direct projections to dopaminergic neurons of the VTA (Nauta et al. 1978). These medium spiny neurons in turn receive direct projections from the PFC, and indirect PFC inputs via the ventral hippocampus, basolateral nucleus of the amygdala and the pedunculopontine tegmentum (Grace, 2000; Herbert et al. 1997; Pennartz et al. 1994), all of which are targets of the PFC. Apart from modulating the activity of mesocortical dopaminergic neurons, dopamine release in the NAc is directly controlled by PFC–NAc projections (Le Moal & Simon, 1991). Therefore, altered PFC activity affecting stress-induced behaviours that are dependent on dopamine neurotransmission in the NAc, as seen in the present study, may occur via several different mechanisms.

Increased spontaneous and amphetamine-induced locomotion (Carter & Pycock, 1980; Pycock et al. 1980a, b), as well as enhanced stress-induced subcortical dopaminergic activity (Deutch et al. 1990) have been described following bilateral depletion of dopamine in the mPFC in rats. However, a number of reports have claimed that bilateral depletion of dopamine in the mPFC did not produce an increase in spontaneous or amphetamine-induced locomotion (Banks & Gratton, 1995; Clarke et al. 1988; Joyce et al. 1983). Interestingly, in the present study, spontaneous activity was not affected but stress-induced behaviours were affected in lesioned rats. Some of these discrepancies might be due to the extent of noradrenergic fibre loss in the PFC during 6-OHDA-induced depletion of dopamine fibres. Noradrenergic terminals are primarily responsible for clearing dopamine from the synaptic clefts in the PFC (Venator et al. 1999). Employing an identical lesioning method as the present study, King & Finlay (1995) found that selective depletion of prefrontal cortical dopamine did not alter basal, amphetamine-evoked or stress-induced dopamine release in the neostriatum and acute stress did not increase locomotor activity in their animals. However, in a subsequent series of studies, King et al. (1997) found a similar depletion of prefrontal cortical dopamine in the same animal species resulted in increased release of dopamine in the shell but not within the core region at baseline and following acute stress in lesioned compared to sham-operated animals. Present results favour the latter observations, but indicate that NAc core may also play a role in mediating stress responsiveness.

Differences in connections, neurochemical composition, synaptic organization and function between the shell and core regions of the NAc have been described (Deutch & Cameron, 1992; Jongen-Reilo et al. 1993; Kalivas & Duffy, 1995; Zahm, 1992). The NAc core is generally considered as part of the motor system whereas the shell is involved mainly in limbic function (Brog et al. 1993; Groenewegen & Russchen, 1984; Zahm & Heimer, 1993). Several observations support efflux of stress-induced dopamine preferentially within the NAc shell (Deutch & Cameron, 1992; Kalivas & Duffy, 1995; King et al. 1997). Using in-vivo microdialysis, King et al. (1997) described that prefrontal cortical dopaminergic activity modulates amphetamine-evoked dopamine efflux in the NAc core, and stress-induced dopamine efflux in the shell. It is possible that effects seen following the high dose of raclopride infused into the core in the present study might be due to diffusion of raclopride from the core region to the adjoining shell.

Reduced prefrontal cortical dopamine neurotransmission may be associated with schizophrenia (Akil et al. 1999; Bogerts et al. 1983; Davis et al. 1991; Heritch, 1990; Franzen & Ingvar, 1975; Ingvar & Franzen, 1974; Kahn et al. 1994; Ribeyre et al. 1994; Weinberger, 1987; Weinberger et al. 1988). In addition, in-vivo imaging data revealed decreased dopamine neurotransmission in the PFC, particularly at D1 receptors, in schizophrenia patients (Abi-Dargham, 2004; Abi-Dargham & Moore, 2003; Okubo et al. 1997). Present results indicate that decreased D1 dopamine receptor neurotransmission in the PFC may contribute to the manifestation of stress-induced subcortical dopaminergic hyperactivity in schizophrenia patients. However, it is important to note that the lesioning methods employed in the present study resulted in ~80% depletion of dopaminergic fibres in the PFC whereas Akil et al. (1999) reported ~35% depletion of dopaminergic fibres in the PFC of schizophrenic brains. Nonetheless, based on the current results as well as the results of others, augmenting D1 receptor function in the PFC in schizophrenia patients may prove beneficial against certain positive symptoms in addition to its potential role in alleviating cognitive deficits.

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Statement of Interest

None.
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