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ORIGINAL INVESTIGATION

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Desipramine attenuates working memory impairments induced by partial loss of catecholamines in the rat medial prefrontal cortex

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Abstract *Rationale:* The density of tyrosine hydroxylase-immunoreactive (TH-IR) axons in the prefrontal cortex of schizophrenic subjects may be reduced by as much as 50% in the deep cortical layers (Am J Psychiatry 156:1580–1589, 1999). Previously, we demonstrated that ~60% loss of TH-IR axons in the rat medial prefrontal cortex (mPFC) decreases local basal and stress-evoked extracellular dopamine (DA) concentrations, suggesting that moderate loss of DA axons in the mPFC is sufficient to alter the neurochemical activity of the remaining DA neurons (Neuroscience 93:497–505, 1999). *Objectives:* To further assess the functional consequences of partial mPFC DA depletion, we examined the effects of 6-hydroxydopamine lesions of the rat mPFC on behavior in a T-maze delayed-response task. We also assessed whether chronic administration of the norepinephrine (NE) uptake inhibitor, desipramine (DMI), attenuates lesion-induced deficits in T-maze performance. Previous research indicates that inhibition of NE transport in the mPFC results in a concomitant increase in extracellular DA and NE. *Results:* Moderate loss of mPFC DA and NE (~50 and 10% loss, respectively) was sufficient to impair delayed-response behavior, in part due to an increase in perseverative responding. Chronic DMI treatment (3 mg/kg delivered via osmotic pumps) impaired performance of control rats but attenuated the deficits in delayed-response behavior in rats previously sustaining loss of mPFC DA and NE (~75 and 35% loss, respectively). *Conclusion:* These data suggest that moderate loss of DA and NE in the prefrontal cortex is sufficient to impair cognitive function, and these behavioral effects are attenuated by inhibition of the NE transporter.

Keywords Norepinephrine · 6-Hydroxydopamine · Delayed response · T-maze

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Introduction

Converging evidence supports an inverted U-shaped relation of mesoprefrontal dopamine (DA) neuron activity and cognitive performance, such that DA receptor stimulation above or below an optimum level gives rise to cognitive deficits (Arnsten 1997; Granon et al. 2000; Zahrt et al. 1997). Dysfunction of the mesoprefrontal DA system has long been thought to contribute to the cognitive deficits associated with schizophrenia (Jaskiw and Weinberger 1992; Robbins 1991). In support of this hypothesis, local application of DA antagonists or DA depletion of the medial prefrontal cortex (mPFC) in experimental animals produces cognitive deficits reminiscent of those seen in schizophrenic subjects (Arnsten et al. 1994; Brozoski et al. 1979; Bubser and Schmidt 1990; Ellenbroek et al. 1996; Granon et al. 2000; Koch and Bubser 1994; Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994). Immunocytochemical analyses of postmortem tissue from schizophrenic subjects have confirmed that the density of prefrontal cortex nerve fibers immunoreactive for tyrosine hydroxylase (TH-IR) and DA transporter protein is reduced by up to 50% (Akil et al. 1999). A decrease in size and number of neuromelanin-containing cell bodies has also been observed in the ventral tegmental area of schizophrenic subjects (Bogerts et al. 1983). A moderate reduction in TH-IR fibers in the rat mPFC is sufficient to reduce basal and stress-evoked extracellular DA concentrations in this brain region (Venator et al. 1999). The present studies examined whether moderate reductions in the DA innervation of the rat mPFC, comparable to that observed in

schizophrenic subjects, are sufficient to induce cognitive deficits as assessed using a T-maze alternation task.

DA agonists attenuate lesion-induced cognitive deficits in animals previously sustaining loss of prefrontal cortex DA (Brozoski et al. 1979) and improve cognitive performance and cognitive activation of cerebral blood flow in the frontal cortex of schizophrenic subjects (Daniel et al. 1989; Daniel et al. 1991; Dolan et al. 1995; Fletcher et al. 1996; Geraud et al. 1987). However, DA agonists also evoke or exacerbate hallucinations and delusions in schizophrenic subjects (Angrist et al. 1985; Angrist et al. 1980; van Kammen et al. 1982), perhaps as a result of increasing activity at subcortical DA receptors. In contrast, norepinephrine (NE) uptake inhibitors have been found to increase extracellular DA in the PFC, while leaving subcortical DA systems largely unaffected (Carboni et al. 1990; Gresch et al. 1995; Moron et al. 2002; Yamamoto and Novotney 1998) These effects are thought to reflect the fact that NE transporters located on noradrenergic axons contribute to clearance of extracellular DA in the NE-rich mPFC, but not in DA-rich/NE-poor subcortical sites. The present studies examined whether chronic administration of the NE transport inhibitor desipramine (DMI) alleviates cognitive deficits induced by partial loss of DA in the rat mPFC.

Experimental procedures

Animals and materials

Male Sprague–Dawley rats (Hilltop, Scottdale, Pennsylvania) were housed singly in wire mesh cages (20×20×30 cm) in a temperature-controlled room (22–23°C). Room lights were on from 8:00 a.m. to 8:00 p.m. Rats were allowed at least 1 week to acclimate to the colony room prior to any treatment. During this time, rodent chow and water were available ad libitum. Procedures for the treatment of rats were approved by the Institutional Animal Care Use Committee at the University of Pittsburgh and Western Washington University using criteria established by the U.S. Animal Welfare Act and the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

6-OHDA lesions of the mPFC

6-Hydroxydopamine (6-OHDA) lesions of the mPFC were performed as previously described (King and Finlay 1995; Venator et al. 1999). Briefly, rats weighing 220–250 g were treated with DMI hydrochloride (25 mg base/kg, i.p.; Sigma Chemical Co., St. Louis, MI) 30 min prior to anesthesia with Equithesin (3 ml/kg, i.p.). Using a stereotaxic instrument (David Kopf Instruments, Tujunga, CA), a glass pipette (tip o.d.=50–100 μm) was positioned in the mPFC at the following coordinates: AP +3.2 and ML ±0.8 mm from bregma, and DV −3.2 mm from dura with a flat skull (Paxinos and Watson 1986). After the pipette was in position for 5 min, 1.0 μg 6-OHDA base (prepared from 6-OHDA hydrochloride; Sigma) in 2 μl of vehicle (0.9% sodium chloride containing 0.03% ascorbic acid) was infused into each site over 5 min using pressure ejection (World Precision Instruments PV820 Pneumatic Picopump, Sarasota, FL). The glass pipette was left in position for an additional 5 min to allow for dispersal of the toxin. After recovering from anesthesia, rats were returned to their cages in the colony room where they remained undisturbed for 1 to 2 weeks.

T-maze delayed-response training and testing

A T-maze was constructed of clear acrylic. The main alley was 90 cm long and 11 cm wide. The initial 30 cm of the main alley constituted the start box and could be partitioned from the remainder of the main alley using a moveable door. The main alley terminated in two arms, each 50 cm long and 10 cm wide, extending at 90° angles to the right and left of the main alley. Moveable doors were located at the start of the main alley and 20 cm from the end of each arm. Reward receptacles, located at the ends of each arm, were positioned below floor level to prevent rats from seeing whether the dish was baited. The walls of the maze were 10 cm in height, and the entire maze was covered by a clear acrylic lid. The floor, walls, and doors of the maze were painted black.

The protocol for the T-maze delayed-response task was based on a previously published method (Thomas and Gash 1988). Briefly, the task consisted of several days of habituation, one-arm adaptation, alternation, and delayed-response trials. During habituation trials (began 1–2 weeks postlesion), rats were placed in the start box of the main alley. The door of the start box was immediately opened, allowing the rat free access to the maze and reinforcers (1/2 of a milk-chocolate chip) located in the receptacles at the end of the arms. On habituation days 1 and 2, rats were confined to the maze for 5 and 3 min, respectively. Subsequent exposures were 2 min each in duration. Daily habituation trials continued until all rats were consuming the chocolate (4–6 days). During one-arm adaptation trials, rats were placed in the start box of the main alley, and the door was immediately opened allowing free access to the main alley and one randomly selected arm, baited with chocolate. Access to the alternate arm was blocked by the removable door. Rats were given six 1-arm adaptation trials per day. Daily one-arm adaptation trials were continued until all rats were consuming the chocolate on all trials (5 days). Alternation trials were comprised of an *information run* and a *choice run.* During the *information run*, rats were placed in the start box, and the door was immediately opened to allow free access to the main alley and 1-arm baited with chocolate. Immediately following consumption of the chocolate, rats were returned to the start box for the initiation of the *choice run*. The door of the start box was immediately opened to allow free access to the main alley and both arms. Only the arm not previously entered on the *information run* was baited. As soon as the front half of the rat's torso was within an arm, the door at the entry was closed, confining the rat to the arm. Regardless of whether the choice run resulted in reinforcement, it was followed by the next information run. Rats performed six such alternation trials per day until the average performance of each group reached a

criterion of 75–80% correct responses on 2 consecutive days (5–8 days). The procedure for delay trials was identical to alternation trials with the exception that a 30-, 60-, or 90-s delay was introduced between the information and test runs. Retention intervals were similar to those used in previously published research examining the role of mPFC in working memory (Aultman and Moghaddam 2001; Bubser and Schmidt 1990; Jentsch et al. 1997). During delays, rats were housed in a cylindrical clear acrylic cage (27 cm diameter×40 cm high). Six delay trials (two of each delay) were conducted daily for 5 days. The order of delay presentations within a day was randomized across trials. Throughout T-maze training and testing, the floor of the maze was wiped with unscented baby wipes between every trial.

All behavioral training/testing was performed during the light phase of the light–dark cycle. Rats were maintained at body weights that were 85–90% of a group of age-matched free-feeding control rats. Following each behavioral training/testing session, rats were weighed, returned to their cages, and given a quantity of rodent chow sufficient to maintain the target body weight. On nontraining/testing days, chow was placed in each cage at a random time during the light phase of the light–dark cycle.

Chronic drug administration

Osmotic pumps (Alzet Model 2ML4; Alza Corporation, Palo Alto, CA) were implanted immediately following the last T-maze habituation trial. Control and lesioned rats (n=13 and 11, respectively) were anesthetized (Equithesin, 3 ml/kg, i.p.), and pumps were implanted subcutaneously on the lateral aspect of the torso, caudal to the left or right forelimb. Osmotic pumps delivered 3 mg DMI base (DMI hydrochloride; Sigma) in 60 μl of 0.9% sodium chloride per day for the remainder of the experiment (~11 mg/kg DMI base per day). Sustained delivery of DMI via osmotic pumps did not result in tissue necrosis commonly seen following repeated subcutaneous injections. Drug-naïve control and lesioned rats were either untreated, or osmotic pumps were implanted to deliver 60 μl of vehicle per day. Because the behavior of untreated and vehicle-treated rats did not differ, data from these groups were combined for analysis of behavior in drug-naive control and lesioned groups (n=24 and 18, respectively).

Tissue preparation and neurochemical analyses

Local application of 6-OHDA produces a rapid and persistent loss of brain catecholamines (Bell et al. 1970; Boyce and Finlay 2005). To determine lesion size in the present study, tissue preparation was performed approximately 14 days after behavioral testing was completed, using previously published methods (King and Finlay 1997). Briefly, rats were decapitated, the brains were removed, and the left and right mPFC were dissected from 1.4-mm-thick coronal sections cut at the level of the mPFC. Tissue samples were weighed, homogenized, centrifuged, and filtered. The resulting supernatant was stored at −80°C until analyzed. DA, NE, and 3,4-dihyroxyphenylacetic acid (DOPAC) concentrations in the supernatant were determined using high-pressure liquid chromatography (HPLC) with electrochemical detection. The assay conditions were similar to those used previously in our laboratory (Venator et al. 1999). Peak separation was accomplished using a reversed-phase C18 column (ESA, Part #70-0636, Chelmsford, MA) and mobile phase (ESA, Part #71-1332). Electrochemical detection was performed using amperometric (Waters, Model 460) or coulometric detection (ESA, Model 5100A). Oxidation peaks produced by analyzing supernatant were compared to those produced by standards of a set concentration. Acquisition and analysis of chromatograms were performed using a computer (Dynamax Macintegrator HPLC Method Manager, Rainin Instrument Co., Emeryville, CA).

Statistical analyses

Tissue DA, DOPAC, and NE concentrations (nanograms per milligram tissue wet weight) in the mPFC of control and 6-OHDA-lesioned rats were analyzed using one-way ANOVAs. Behavioral data were analyzed using one-way ANOVAs, two-way repeated measures ANOVAs, or independent samples t tests. Degrees of freedom appropriate to the Greenhouse–Geisser F test were used in evaluating the results of repeated measures ANOVAs (Kirk 1982); however, the unadjusted degrees of freedom are reported. The level of significance for all ANOVAs was $P \leq 0.05$. Following a significant overall F value, pairwise comparisons were performed using "layered" Bonferroni t tests (Darlington 1990). Data analyses were performed using SPSS for Windows (SPSS Inc., Version 10.1, Chicago, IL). All data are presented as mean±SEM.

Results

Effects of 6-OHDA lesions of the mPFC on T-maze performance

Local infusions of 6-OHDA decreased absolute DA and NE concentrations in the mPFC [Table 1; $t(39)$=7.00 and 2.03, respectively]. Based on the percentage loss of DA in the mPFC, the lesioned group was divided, a posteriori, into two groups via a median split. This split resulted in lesioned groups characterized by ~50 and 85% loss of tissue DA concentrations relative to control [smaller and larger lesion groups, respectively; $F(2,38)$=40.3; $t(30)$=4.6 and 7.6, respectively]. In rats sustaining larger loss of DA, tissue NE and DOPAC content were also reduced relative to controls [$F(2,38)$=3.7 and 3.4, respectively; $t(30)$=2.8 and 2.4, respectively]. In contrast, tissue NE and DOPAC concentrations in rats sustaining smaller lesions were not significantly different from control values.

Control and smaller and larger lesioned rats exhibited similar behavior during alternation training. All groups

Table 1 Tissue DA, NE, and DOPAC in the mPFC of control and lesioned rats

	DA	NE (ng/mg tissue)	DOPAC
Control (n=23[a])	0.07±0.01	0.23±0.03	0.04±0.01
Lesion (n=18)	0.02±0.00*	0.14±0.02* (% of control[b])	0.02±0.01
Smaller lesion (n=9)	47±3*	88±20	71±13
Larger lesion (n=9)	14±2*	44±8*	51±9*

DA dopamine, *NE* norepinephrine, *DOPAC* 3,4-dihyroxyphenylacetic acid

*Significantly different from control (independent samples t tests with layered Bonferroni correction in the case of multiple comparisons, $P \leq 0.025$–0.05)

[a]Samples of one control rat were omitted from the neurochemical analyses due to contamination. Local infusions of 6-OHDA reduced absolute DA and NE concentrations in the mPFC

[b]Based on the percentage loss of tissue DA, the lesioned group was divided, a posteriori, into two groups via a median split. Relative to the control group, mPFC DA content was reduced in the smaller and larger lesion group. Tissue NE and DOPAC concentrations were also reduced in rats sustaining larger, but not smaller, loss of DA. Data are presented as group mean±SEM

achieved performance accuracies of 75–80% correct responses and made choices within ~4.0 s of being released from the start box (Fig. 1). Performance on the final day of alternation training was compared to that observed when 30-, 60-, and 90-s delays were imposed between information and choice runs (Fig. 2a). Control rats and rats sustaining larger lesions exhibited a decline in response accuracy under the 90-s delay condition [Control: $F(3,69)=4.8$ and $t(23)=4.5$; Larger lesion: $F(3,24)=4.5$ and $t(8)=2.5$], whereas rats sustaining smaller lesions exhibited fewer percent correct responses under 30-, 60-, and 90-s delay conditions [$F(3,24)=9.7$; $t(8)=4.0$, 4.8, and 4.9, respectively]. Delay trial performance was also assessed for lesion-induced alterations in the latency to select an arm (latency timing began when the start box door opened and ended when a choice arm was selected; latencies were determined for both correct and incorrect choices) and perseverative responding (the number of consecutive selections of the same arm divided by the number of consecutive selections of the same arm required to achieve 100% accuracy). Because latencies and perseveration scores did not vary as a function of delay duration, data were collapsed across this variable. Similarly, data were collapsed across the variable of lesion size since rats sustaining smaller and larger lesions exhibited similar choice latencies (2.7±0.4 and 2.3±0.2 s, respectively) and perseveration scores (1.8±0.2, both groups). Partial depletion of DA in the rat mPFC did not alter delay trial choice latencies (Fig. 2b). However, relative to controls, lesioned rats exhibited a 20% increase in perseverative responding during delay trials [Fig. 2c; $t(40)=2.1$].

Effects of chronic DMI on T-maze performance in rats previously sustaining 6-OHDA lesions of the mPFC

The effects of chronic DMI (3 mg/day) on delayed-response behavior were assessed in control rats and rats previously sustaining 6-OHDA lesions of the mPFC. Neurochemical and behavioral data from the DMI-treated groups were compared to those obtained from the drug-naïve control and lesioned rats presented above (as noted in "Experimental procedures", these rats were either untreated or received vehicle infusions). DA concentrations in the mPFC of DMI-treated lesioned rats were reduced by ~75% relative to DMI-treated control rats (0.02±0.01 and 0.08±0.01 ng/mg tissue, respectively; $t(20)=4.6$], and NE concentrations were reduced by ~35% [0.20±0.08 and 0.31±0.10 ng/mg tissue, respectively; $t(20)=2.9$]. DA and NE concentrations in the mPFC of DMI-treated control and lesioned rats did not differ from drug-naïve control and lesioned rats, respectively, indicating that prior chronic administration of DMI did not impact the quantification of lesion size. Based on the percentage loss of mPFC DA, the DMI-treated lesioned group was divided, via a median split, into a smaller and larger lesion group (~50 and 90% loss of tissue DA). Because subsequent statistical analyses revealed that the behavior of DMI-treated lesioned rats did not vary

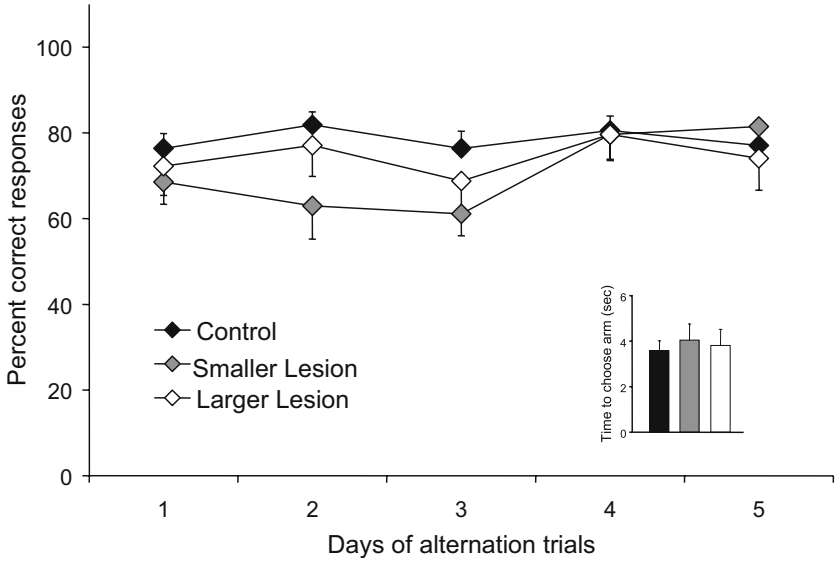

Fig. 1 Effects of 6-OHDA lesions of the MPFC on performance during the last 5 days of T-maze alternation trials (n=24 control, n=9 lesion/group). Percent correct responses and choice latency (*inset*) were similar in control and lesioned rats. Data are presented as group mean±SEM

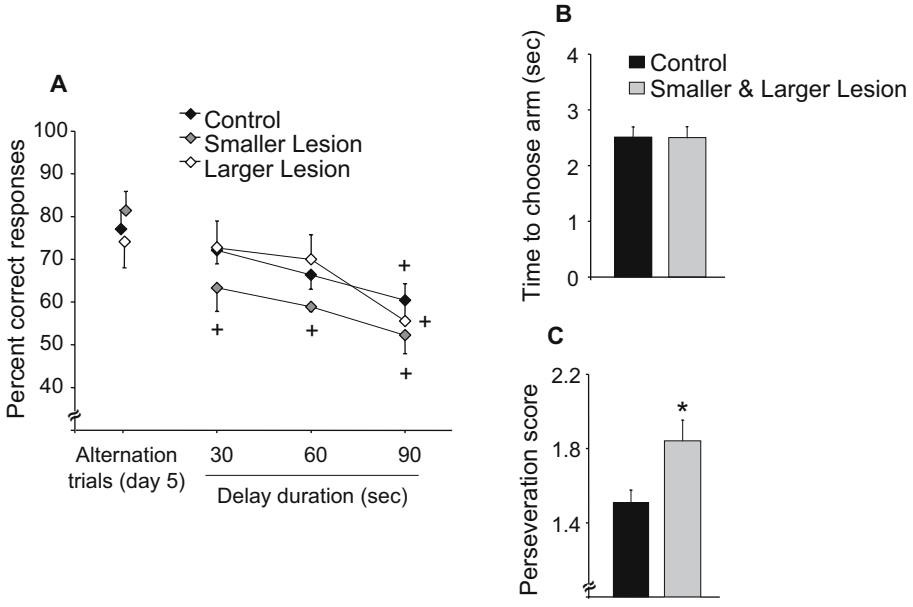

Fig. 2 Effects of 6-OHDA lesions of the mPFC on performance accuracy, choice latency, and perseverative errors during T-maze delayed-response trials (n=24 control, n=9 lesion/group). **a** Relative to the final day of alternation trials, rats sustaining smaller lesions exhibited impaired performance under 30-, 60-, and 90-s delays. Control and larger lesion rats exhibited impaired performance only under the 90-s delay condition. **b** Choice latencies and **c** perseverative errors were collapsed across delay and lesion size. Partial loss of mPFC DA did not affect choice latency but increased perseverative responding. Data are presented as group mean±SEM. †Significantly different from corresponding within-group alternation trials (paired samples t tests with layered Bonferroni correction, $P \leq 0.017$–0.05). *Significantly different from control (independent samples t test, $P \leq 0.04$)

significantly as a function of lesion size, behavioral data were collapsed across this variable for analysis.

Average percent correct responses and choice latencies over the last 5 days of alternation trials were calculated for DMI-treated control and lesioned rats and drug-naïve control and lesioned rats. DMI-treated control rats exhibited fewer percent correct responses than drug-naïve control rats, naïve-lesioned rats, and DMI-lesioned rats [Fig. 3a; $F(3,61)=4.3$); $t(35)=2.5$, $t(29)=2.1$, and $t(22)=2.7$, respectively]. DMI-treated control rats sometimes failed to select an arm within 90 s of initiation of a choice run (~1 trial/day). Response failures were coded as an error in the data presented in Fig. 3a. When trials associated with response failures were omitted from the data set, performance of DMI-treated

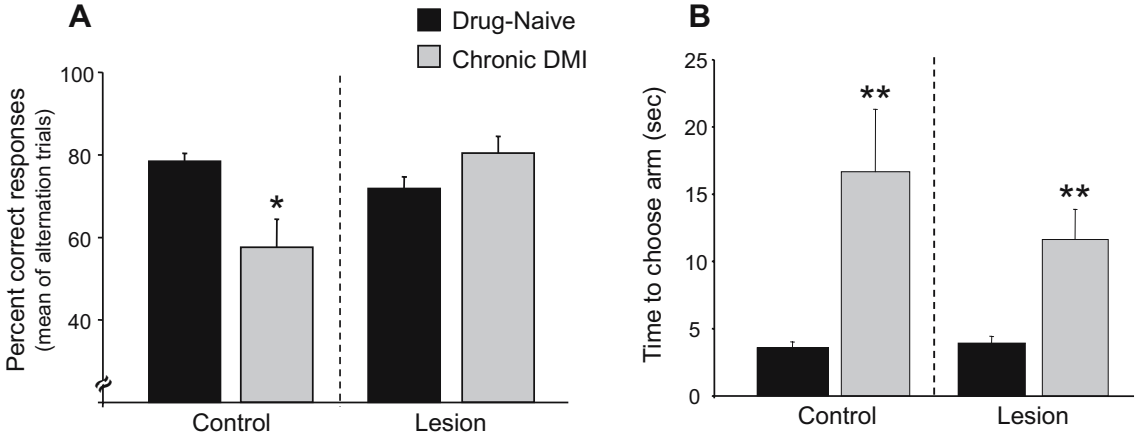

Fig. 3 Effects of chronic DMI (3 mg/day delivered via osmotic pumps) on percent correct responses (**a**) and choice latencies (**b**) during T-maze alternation trials in control rats and rats previously sustaining ~75% loss of DA in the mPFC (n=13 DMI-control, n=11 DMI-lesion, n=24 drug-naïve control, n=18 drug-naïve lesion). Because the behavior of DMI-treated lesioned rats did not vary significantly as a function of lesion size, data were collapsed across this variable. Average percent correct responses and choice latencies were calculated for each treatment condition, collapsing across 5 days of alternation trials. DMI-control rats exhibited fewer percent correct responses than drug-naïve control rats, naïve-lesioned rats, and DMI-lesioned rats (n=13, 24, 18, and 11, respectively). Chronic DMI increased choice latency in control and lesioned rats, relative to the corresponding drug-naïve condition. Data are presented as group mean±SEM. *Significantly different from all other treatment conditions (independent samples t tests with layered Bonferroni correction, $P \leq 0.012$–0.05). **Significantly different from corresponding naïve-control and naïve-lesion group (independent samples t tests with layered Bonferroni correction, $P \leq 0.012$–0.05)

control rats (73±5% correct responses) did not differ significantly from that of the other treatment conditions. Relative to their drug-naïve comparison groups, DMI-treated control and lesioned rats also exhibited increased choice latencies during alternation trials [Fig. 3b; $F(3,61)=12.0$; $t(32)=4.4$ and $t(27)=4.2$, respectively].

Following alternation trials, the performance of drug-naïve and DMI-treated control and lesioned rats was evaluated under conditions of 30-, 60-, and 90-s delays between information and choice runs (Fig. 4). Consistent with their performance on alternation trials, DMI-treated control rats exhibited 50–60% correct responses during delay trials. This level of performance was significantly below that of drug-naïve control rats and DMI-treated lesioned rats [group main effect: $F(3,51)=3.3$; $t(35)=2.33$ and $t(22)=2.55$, respectively]. Relative to their respective within-group alternation trials, drug-naïve control rats and DMI-treated lesioned rats exhibited impaired performance only under the 90-s delay condition [$t(23)=4.51$ and $t(10)=2.43$]. During delay trials, DMI-treated control rats again sometimes failed to select an arm within 90 s of initiation of a choice run (~2 trials/day). Response failures were coded as an error in the data presented in Fig. 4. When trials associated with response failures were omitted from the data set, performance of DMI-treated control rats did not differ from that of DMI-treated lesioned rats (averaging 64±4% and 69±4% correct responses across all delay trials, respectively).

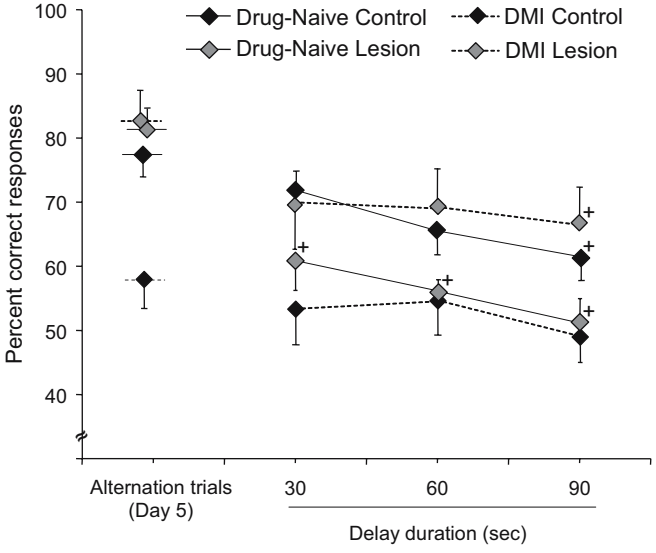

Fig. 4 Effects of chronic DMI (3 mg/day delivered via osmotic pumps) on performance of control rats and rats previously sustaining ~75% loss of DA in the mPFC in a T-maze delayed-response task (n=13 DMI-control, n=11 DMI-lesion, n=24 drug-naïve control, n=9 drug-naïve lesion). Delayed-response performance of DMI-treated control rats was impaired relative to drug-naïve control rats and DMI-treated lesioned rats. Relative to their respective within-group alternation trials, naïve-control rats and DMI-lesioned rats exhibited impaired performance only under the 90-s delay condition. In DMI-control rats, delay trial accuracy did not differ from that observed during alternation trials and remained significantly below that of naïve-control and DMI-lesioned rats. Data are presented as group mean±SEM. $^{+}$Significantly different from corresponding within-group alternation trials (paired samples t tests with layered Bonferroni correction, $P \leq 0.017$–0.05)

Discussion

Effects of 6-OHDA lesions of the mPFC on T-maze performance

Postmortem analyses have revealed a modest decrease in TH-IR nerve terminals in the PFC of schizophrenic subjects, approaching a maximum of ~50% in the deep cortical layers (Akil et al. 1999). The goal of the present study was to determine whether, in rats, this structural abnormality is sufficient to impair the functional capacity of the remaining mesoprefrontal DA neurons. Our results indicate that ~50% loss of tissue DA content in the rat mPFC impaired performance on T-maze delayed-alternation trials. During acquisition of the task, performance accuracy of rats sustaining ~50% loss of mPFC DA was similar to that of control rats. However, when delays of 30-, 60-, and 90-s were introduced between the information and choice runs, lesioned rats exhibited greater performance deficits than controls. The lesion-induced decrease in accuracy was associated with increased perseverative responding. The present data are consistent with earlier reports indicating that delayed-response behavior is impaired in nonhuman primates and rats sustaining ≤50% loss of PFC DA (Roberts et al. 1994; Stam et al. 1989). In contrast, results of an early study suggested that ~50% loss of PFC DA has no effect on spatial delayed-response behavior in nonhuman primates (Brozoski et al. 1979). The lack of effect of moderate lesions in the latter study may have been related to the use of a within-subject experimental design in which subjects were trained and tested on the delayed-response task prior to lesioning. Moderate loss of DA terminals in the rat mPFC has been found to impair the neurochemical activity of this system (Venator et al. 1999). In the latter study, absolute basal and stress-evoked extracellular DA concentrations in the mPFC were reduced following ~65% loss of TH-IR axons in the mPFC. Thus, relatively moderate loss of PFC DA terminals may be sufficient to alter local extracellular DA concentrations and, in turn, behaviors modulated by this system.

Mesoprefrontal DA neurons appear to play a role in several aspects of cognitive function including working memory and attention (Chudasama and Robbins 2004; Granon et al. 2000). Additional studies are required to identify the specific cognitive dysfunction associated with deficits in T-maze delayed-response behavior observed in the present study. Lesion-induced motor and/or motivation deficits may also contribute to poor performance in a T-maze task. However, the observation that lesions failed to affect response latencies (interval between start-box door opening and an arm choice) and reward retrieval during information runs provides initial evidence to the contrary. Lesioned rats exhibiting impaired T-maze performance also sustained ~10%, albeit nonsignificant, loss of mPFC NE. As suggested by other investigators, the role of NE (Arnsten 1997) and DA–NE interactions (see below for further discussion) in these behavioral deficits must also be considered.

In the present study, rats sustaining ~85% loss of mPFC DA and ~50% loss of NE performed similar to control rats

during all phases of T-maze testing. This finding is consistent with the observation that ~80% loss of mPFC DA is associated with near-normal basal and stress-evoked extracellular DA in this brain region (Venator et al. 1999; Sleipness et al. 2001). Normalization of local extracellular DA in rats sustaining ~80% loss of mPFC DA may be due, in part, to partial loss of NE nerve terminals and the associated NE transporters, thereby reducing uptake of extracellular DA into NE neurons (Venator et al. 1999). Despite pretreatment with a NE uptake inhibitor, local infusions of 6-OHDA frequently induce a decrease in mPFC NE content. Although the present data are not consistent with previous reports that PFC DA depletions in this range impair delayed response in nonhuman primates (Brozoski et al. 1979; Roberts et al. 1994), subjects in the latter studies also sustained larger NE depletions than our animals. Differences in the relative loss of NE and DA may be critical in determining the effects of 6-OHDA lesions of the PFC on delayed-response behavior. In fact, lesions of the cortical NE innervation have been shown to alter other behavioral effects of PFC DA depletions (Ravard et al. 1990; Taghzouti et al. 1988). As with lesion studies, results of studies using acute pharmacological manipulations generally support a role for mPFC DA neurons in working memory that is much more complex than the originally hypothesized direct relationship between PFC D_1 receptor activation and working memory (Arnsten 1997; Romanides et al. 1999; Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994; Seamans et al. 1998; Williams and Goldman-Rakic 1995; Zahrt et al. 1997). Investigators have begun to consider the importance of basal DA tone and interactions between PFC DA and other neurotransmitter systems, such as NE-containing neurons, in the modulation of cognitive function (Granon et al. 2000; Romanides et al. 1999; Verma and Moghaddam 1996). In agreement with the latter trend, results of the present study suggest that DA–NE interactions in the mPFC play an important role in regulating working memory function.

Effects of DMI on T-maze performance of control rats and rats previously sustaining 6-OHDA lesions of the mPFC

The present study examined the effects of the NE uptake inhibitor DMI (3 mg/kg/day delivered via osmotic pumps) on T-maze delayed-response behavior in control rats and rats previously sustaining 6-OHDA lesions of the mPFC. Chronic DMI impaired the performance of control rats on alternation and delay trials. In this case, performance deficits were due to rats failing to make an arm selection on the choice runs of alternation and delay trials. DMI-treated control rats appeared to be motivated to perform the task in that they did retrieve the reinforcer during information runs. Thus, it is unlikely that the disruption in performance is due to entirely to a sedative effect of the drug. We are aware of only one study examining the effects of a NE uptake inhibitor on normal cognition. In that study, acute administration of a NE uptake inhibitor enhanced working memory in normal humans (Rammsayer et al. 2001). In contrast, results of studies using direct-acting agonists and antagonists suggest that NE acts at α_1- and α_2-receptors in the PFC to impair and facilitate, respectively, working memory in intact rodents and primates (Arnsten et al. 1999; Avery et al. 2000; Birnbaum et al. 1999; Coull et al. 1995; Franowicz and Arnsten 1998; Franowicz et al. 1999; Li and Mei 1994; Mao et al. 1999; Sawaguchi 1998). It is possible that the cognitive deficits observed in the present study are due to DMI-induced increases in mPFC NE, biasing the system in favor of α_1- over α_2-receptor-mediated events. However, DMI also increases extracellular DA in the mPFC of naïve rats (Carboni et al. 1990; Gresch et al. 1995; Yamamoto and Novotney 1998). It has been suggested that the relationship between mPFC DA and cognitive function assumes an inverted U-shaped function such that DA receptor stimulation above or below an optimum level gives rise to cognitive deficits (Arnsten 1997; Granon et al. 2000; Zahrt et al. 1997). Thus, DMI-induced increases in extracellular DA may also have contributed to cognitive impairments in our drug-treated control rats.

Whereas chronic DMI impaired the behavior of control rats, drug treatment attenuated cognitive deficits produced by partial depletion of mPFC DA and NE (~75 and 35%, respectively). Differential effects of DMI on cognitive performance of control and lesioned rats do not appear to be related to a confounding influence of the drug on motor behavior since DMI increased choice latencies (interval between start-box door opening and an arm choice) in both groups. Both direct and indirect NE agonists have been reported to alleviate cognitive dysfunction thought to be due to reduced cortical noradrenergic transmission. For example, NE uptake inhibitors attenuate cognitive deficits in individuals suffering from depression and attention deficit hyperactivity disorder (Ferguson et al. 2003; Popper 2000). In experimental animals, deficits in cognitive function associated with aging, chronic reserpine, or 6-OHDA lesions of the PFC are reduced by α_2-receptor agonists administered systemically or directly into the PFC (Arnsten et al. 1988; Arnsten and Goldman-Rakic 1985; Cai et al. 1993; Carlson et al. 1992; Franowicz et al. 1999). Results of the present study suggest that NE uptake inhibitors are effective in reducing cognitive deficits associated with a more selective loss of DA in the mPFC, as may be the case in schizophrenia. DMI-induced attenuation of the lesion-induced deficits in T-maze behavior may be related to drug-induced increases in DA and/or NE. In fact, systemic DMI has been found to increase extracellular DA and NE in the mPFC of rats previously sustaining similar lesions to those described in the present study (Sleipness et al. 2001). Although DA agonists attenuate cognitive deficits in schizophrenic subjects (Daniel et al. 1991), the risk of drug-induced psychoses prohibits their use in the clinical population. The psychotomimetic effects of the direct and indirect acting DA agonists may be due to drug-induced alterations in activity of subcortical DA systems. Because DMI increases extracellular DA and NE concentrations in

the mPFC while leaving subcortical DA largely unaffected, this and other NE uptake inhibitors may be useful adjuncts in the treatment of cognitive abnormalities associated with schizophrenia.

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