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6-Hydroxydopamine Lesions of the Prefrontal Cortex in Monkeys Enhance Performance on an Analog of the Wisconsin Card Sort Test: Possible Interactions with Subcortical Dopamine

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The effects of 6-hydroxydopamine lesions of the prefrontal cortex in monkeys were investigated on two cognitive tests of prefrontal function, spatial delayed response, and attentional set shifting. The latter test provided a componential analysis of the Wisconsin Card Sort Test, a commonly used clinical test of frontal lobe function in man. Acquisition of a visual compound discrimination requiring a shift of attention from one dimension to another (extradimensional shift), for example, shapes to lines, was significantly improved. This enhancement was behaviorally specific in that there were no effects on acquisition of a discrimination that required the continued maintenance of an attentional set toward one particular dimension (intradimensional shift), nor any effects on a series of visual or spatial discrimination reversals that involved the repeated shifting of responding between two exemplars from the same dimension. In contrast, spatial delayed response performance was impaired, in agreement with previous results. Neurochemical measures showed a marked depletion of dopamine limited to the prefrontal cortex and a smaller loss of prefrontal noradrenaline. This was accompanied by a long-term adaptive change in the striatum such that extracellular dopamine in the caudate nucleus, as measured by *in vivo* microdialysis, was elevated in response to potassium stimulation as long as 18 months postsurgery. It is proposed that attentional set shifting is mediated by a balanced interaction between prefrontal and striatal dopamine, and that elevated dopamine contributes to the improvement in attentional set-shifting ability. This interpretation is consistent with the impairment in attentional set-shifting ability observed in patients with Parkinson's disease or with damage to the frontal lobes using the same test as used here for infrahuman primates.

[Key words: attentional set, set shifting, spatial delayed response, prefrontal cortex, striatum, dopamine, Parkinson's disease, schizophrenia]

A disruption of cognitive functions normally associated with damage to the prefrontal cortex has been reported in a number of neurodegenerative disorders including Parkinson's disease (PD) (Lees and Smith, 1983; Taylor et al., 1986; Canavan et al., 1989) and progressive supranuclear palsy (PSP) (Albert et al., 1974; Pillon et al., 1986; Grafman et al., 1990). In the absence of structural pathology within the prefrontal cortex in these disorders, such cognitive dysfunctions may be attributed to either a disturbance of *prefrontal cortical modulation,* as a result of the degeneration in the monoaminergic (Scatton et al., 1983; Javoy-Agid, 1987) and cholinergic (Dubois et al., 1983; Perry et al., 1985) projections to prefrontal cortex, or a disruption of *prefrontal cortical output,* due to the loss of the dopaminergic innervation of the striatum (Bernheimer et al., 1973), a major output pathway of the prefrontal cortex.

Evidence that catecholaminergic and cholinergic projections to prefrontal cortex may play an important role in cognitive functioning comes primarily from experimental studies in animals where relatively selective lesions of these projection pathways have been made. For example, dopamine loss in the prefrontal cortex of the monkey leads to as great a deficit in performance on a spatial delayed response test (Brozoski et al., 1979), as does ablation of the prefrontal cortex itself (Mishkin, 1957; Gross and Weiskrantz, 1962). The relevance of these findings to the behavioral impairments of patients with neurodegenerative disorders has been demonstrated by using these tests in the clinic, hence allowing direct comparison of the effects of frontal lobe manipulations in experimental animals with neurodegenerative conditions (Lueck et al., 1990).

An analogous approach is used in the present study, in which monkeys are tested on a paradigm derived from a standard clinical test of frontal lobe function, the Wisconsin Card Sort Test (WCST). This requires subjects to shift their "attentional set" from one perceptual dimension of a complex stimulus to another, for example, from color to shape (Milner, 1963, 1982), and patients with frontal lobe damage are known to perseverate in sorting according to the earlier category. By "attentional set" is meant the predisposition to respond to particular stimulus dimensions as a result of pretraining. Difficulties in performance on this test have also been reported in patients with PD (Canavan et al., 1989; Taylor et al., 1986) and PSP (Pillon et al., 1986). Therefore, we have developed a paradigm that not only can be used to study attentional set-shifting ability in both humans and monkeys alike, but also enables the various cognitive processes that underlie successful performance on the WCST to be studied independently of one another.

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A detailed account of the attentional set-shifting paradigm has been described elsewhere (Roberts et al., 1988), but in brief, the paradigm is based on studies of intradimensional and extradimensional shifts used to examine "attentional set" in humans (Eimas, 1966) and other animals (Shepp and Schrier, 1969; Durlach and Mackintosh, 1986). If performance on a visual compound discrimination, in which the subject is required to maintain an attentional set of the previously relevant dimension, that is, color (intradimensional shift, IDS), is superior to performance on a compound discrimination in which the subject is required to shift attentional set to the previously irrelevant dimension, that is, shape (extradimensional shift, EDS), then this is taken as evidence that the subject had developed an attentional set of color. Clearly, by studying these two types of shift in discrimination learning it is possible not only to study attentional set shifting but also to acquire an independent measure of the ability to develop and maintain an attentional set that is lacking in the WCST.

The psychological specificity of the attentional set-shifting paradigm has been confirmed by the selective impairment of patients with frontal lobe damage on the EDS but not the IDS stage of the test (Owen et al., 1991). Moreover, the finding that patients with damage to the temporal lobe or to the hippocampus and amygdala are unimpaired supports its anatomical specificity. When the paradigm has been used to test patients with PD it has been found that EDS performance is sensitive to all stages of PD (Owen et al., 1992), including newly diagnosed patients prior to medication (Downes et al., 1989).

In order that the test can be administered to both monkeys and man, an automated procedure is used in which stimuli are presented on a touch-sensitive video display unit (VDU). The compound stimuli used vary along two abstract dimensions and comprise white lines superimposed over blue-filled shapes. In each of a series of discrimination tests the subject must learn to attend to just one of the dimensions and to choose one particular exemplar from that dimension, for example, the blue-filled triangle in Figure 1*b*. In this example, an IDS would then involve choosing the blue-filled L (Fig. 1*c*) while an EDS would involve choosing the white parallel lines (Fig. 1*e*). When tested with this procedure, monkeys show qualitatively similar patterns of performance to man, namely, superior IDS performance compared with EDS performance (Roberts et al., 1988).

A recent study investigated the performance of marmosets on this paradigm that had received a lesion of the cholinergic innervation to prefrontal cortex similar in extent to that seen in PD (Roberts et al., 1992). Although the lesion resulted in transient impairments in discrimination learning and long-lasting impairments in reversal learning, performance on the IDS and EDS was unaffected. Thus, cortical cholinergic loss is unlikely to account for the attentional set-shifting deficit in PD. Given, however, that certain functions of the prefrontal cortex appear to be critically dependent upon the mesocortical dopamine (DA) input (Goldman-Rakic, 1992), and this is also reduced in PD, the present study investigated the effects of lesioning the prefrontal DA system on performance of marmosets in the attentional set-shifting paradigm. We also tested the animals on a spatial delayed response task to facilitate comparison with other studies of prefrontal DA function (Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1991).

Marmosets were pretreated with a noradrenergic and serotonergic uptake blocker immediately prior to surgery in order to deplete prefrontal DA selectively. As such a lesion can result in an upregulation of subcortical DA activity in rats (Pycock et al., 1980; Glowinski et al., 1988; Rosin et al., 1992), the present study compared DA release in the striatum of lesioned monkeys to that of sham-operated controls using *in vivo* microdialysis.

Materials and Methods

Subjects

Twenty common marmosets (*Callithrix jacchus*) (8 females and 12 males) obtained from the Clinical Research Center, Harrow, and of mean age 15 months, were used in this study. They were housed in either sibling pairs or unisex pairs. All animals were given access to 2 hr of water and fed 20 gm of MP.E1 primate diet [Special Diet Services (SDS), Withams, Essex, UK] and two pieces of carrot in the late afternoon after the daily session of behavioral testing. The diet was supplemented at weekends with fruit, eggs, marmoset jelly (SDS), and peanuts.

Apparatus

A specially designed automated apparatus, situated in a dark room, was used in this study. The animal sat in a Perspex transport cage, which was locked into position in a wooden-sound attenuated box. One side of the transport cage could be removed to expose the screen of a color, high-resolution VDU (model 1440, Microvitec, Bradford, UK). The VDU was fitted with a Microvitec Touchtec 501 touch-sensitive screen for monitoring responses by the marmoset, which was able to view the screen and reach out and touch it through a vertical array of metal bars. A metal spout was attached centrally to the vertical bars for the delivery of liquid reinforcement (ice-cold banana milkshake) from a peristaltic pump. Licking was detected by interruptions of an infrared beam that ran just in front of the spout. Two loud speakers (R. S. components, parts 249–429) were situated to the left and right of the animal on Perspex panels attached to the sides of the touch screen. The testing box was illuminated with a 3 W bulb suspended from the roof in which was drilled a hole covered with tinted Perspex, enabling observation of the animals with the use of a video camera. An illustration of this apparatus can be found as Figure 1 of Roberts et al. (1988).

Visual stimuli were generated by an Acorn BBC+ microcomputer and presented on the screen of the VDU. These consisted of blue-filled shapes (32 mm high and 32 mm wide at their broadest point) and white lines (38 mm high). The microcomputer also controlled the experimental contingencies and the recording of the location and latency of responses to the screen, the latency to lick, and the number of licks by programs written in SPIDER control language (Paul Fray Ltd., Cambridge, UK).

Spatial delayed response performance was measured in a specially designed "hand-testing" apparatus. The monkeys sat in their Perspex transport cage and when one side of it was removed they were able to look through a Perspex window (22 × 7.5 cm). Below the window was a narrow opening that extended almost the full width of the window (21 × 2 cm) through which the subject could reach. On the outside, at the base of the opening, was a Perspex shelf (24 × 4 cm) that contained a central groove through which test boxes could be screwed in various positions. For the delayed response study, two wooden test boxes were used (2 × 2 × 2 cm) that were open on only one side and positioned on the far right and far left of the shelf. During testing, a narrow Perspex screen was placed across the opening during the "sample" stage to prevent the animal from reaching through, while enabling the animal to see the reinforcer being hidden in one or other of the boxes. There then followed a delay period in which an opaque screen was lowered. At the "choice stage," both screens were raised so that the monkey could reach through and choose one of the test boxes.

Locomotor activity was tested in a modified marmoset home cage equipped with eight parallel beams perpendicular to the long axis of the cage and two beams running parallel to the two perches. Interruption of any of the beams resulted in an incremental count, registered by on-line input to a CUBE system 10 microprocessor (Control Universal, Cambridge, UK). [For a detailed description of the apparatus, see Roberts et al. (1988).]

Touch-screen training

All animals were trained to touch a square stimulus presented either to the right or left of the center of the screen. [Full details of the training procedure have been reported in Roberts et al. (1992).]

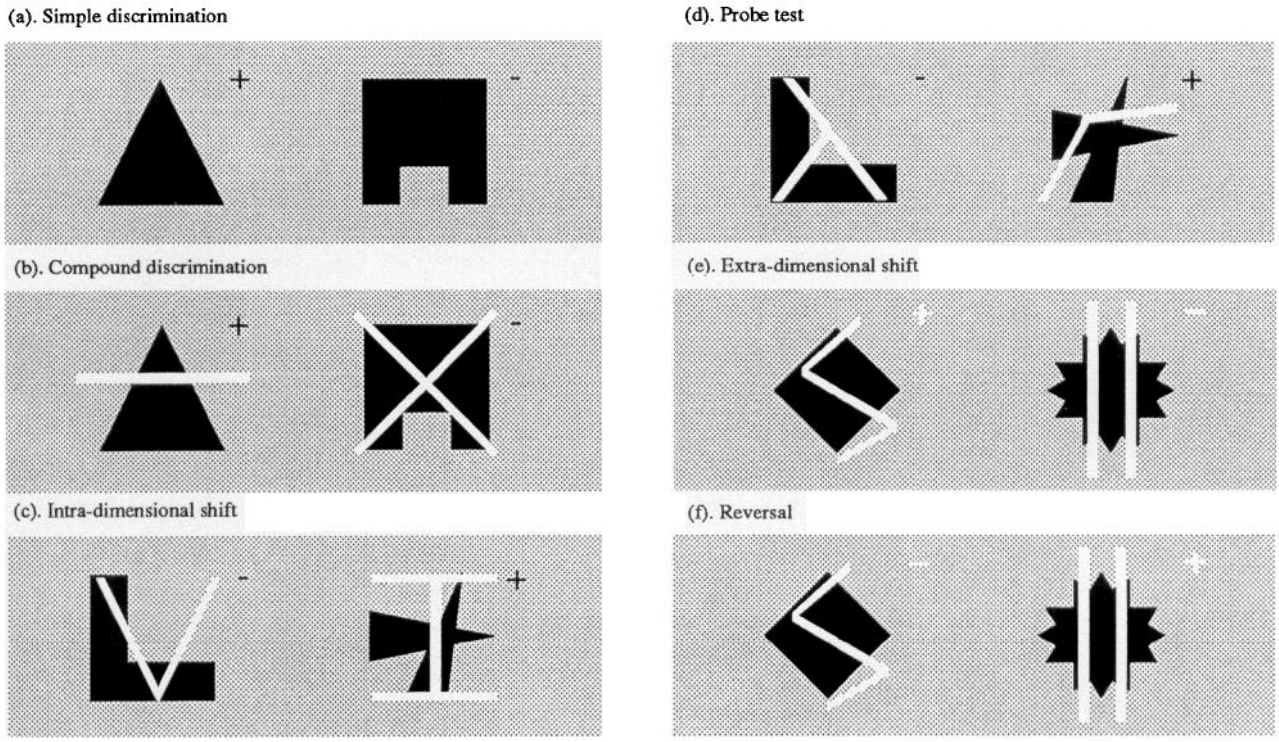


Figure 1. The shape and line exemplars used for the various stages of the attentional set-shifting paradigm. In this example the dimension of "shape" is relevant in all the discriminations except that requiring an EDS and reversal. In any one trial of a compound discrimination a shape exemplar may be paired with one or other of the line exemplars. Correct and incorrect choices are indicated by the *plus* (+) and *minus* (−), respectively. *Black typeface* specifies that "shape" is the relevant dimension while *white typeface* specifies that "line" is the relevant dimension.

Preoperative training

All subjects were trained on a series of simultaneous visual discriminations in which a response to one of two stimuli resulted in the onset of a tone signaling the availability of reinforcement while a response to the other resulted in a 5 sec time-out period, during which the houselight was turned off. A response to either stimulus terminated both of them and ended the trial. If an animal failed to lick within 5 sec following the onset of the signal for reinforcement, the trial was terminated. Subjects were tested for 60 trials/d until they reached a criterion of 90% correct in a single session. The following session they were then presented with a new discrimination.

Initially, the stimuli varied in only one dimension, namely, blue-filled shapes or white lines (Fig. 1*a*). The second, alternative dimension was then introduced to form compound stimuli comprising white lines superimposed over blue-filled shapes (Fig. 1*b*). On any one trial a white line was paired with one or other of the blue-filled shapes. To succeed, an animal had to continue responding to the previously correct stimulus, ignoring the presence of the new, irrelevant dimension. A further two-compound discriminations were presented prior to surgery consisting of new exemplars from each of the two dimensions. For those animals trained on blue-filled shapes, shapes remained correlated with reinforcement while for those animals trained on white lines, lines remained correlated with reinforcement. The second dimension introduced remained irrelevant to the discrimination.

Half of the animals from the shape group and half of the animals from the line group were designated experimental subjects (groups: lesion/shape, $n = 5$ and lesion/line, $n = 6$) while the remaining animals were designated sham-operated controls (groups: control/shape, $n = 4$ and control/line, $n = 5$).

Surgery

All animals were premedicated with the monoamine oxidase inhibitor pargyline (Sigma; 50 mg/kg, i.p.) and 20 min later anesthetized with pentobarbitone (0.15 ml of a 60 mg/ml solution, i.p.). They were then placed in a stereotaxic frame (David Kopf, Tujunga, CA) using a head holder with incisor and zygoma bars specially modified for the marmoset. The dopaminergic innervation of the prefrontal cortex was destroyed by injecting 2 μl of a 6 μg/μl solution of 6-hydroxydopamine hydrobromide (6-OHDA; Sigma; in 0.01% ascorbic acid) bilaterally into 15 sites within the prefrontal cortex. Infusions were made over 100 sec through a stainless steel cannula (30 gauge) attached to a 5 μl precision sampling syringe (SGE, Baton Rouge, LA). The stereotaxic coordinates used were AP +16.5, L ±1.5, ±3.0, and ±5.0; AP +18.5, L ±1.0, ±2.5, and ±4.0 (after Stephan et al., 1980). As there was considerable individual variation in the thickness of the frontal pole the vertical coordinates were calculated independently for each marmoset. At each of the above sites injections were made 0.5 mm above the floor of the skull, 0.5 mm below the surface of the dura, and, depending upon the thickness of the cortex, a third injection was made equidistant between the two. To protect the noradrenergic and serotonergic innervations of the prefrontal cortex from the toxic effects of 6-OHDA, all marmosets were pretreated with the noradrenaline (NA) uptake blocker talsupram (Lundbeck, Copenhagen, Denmark; 15 mg/kg, s.c.) (Arnt et al., 1985) and the serotonin uptake blocker citalopram (Lundbeck; 5 mg/kg, s.c.) (Hyttel, 1982) 30 min prior to the injections of 6-OHDA. In the pilot study, full protection of noradrenaline was only achieved with a dose of 20 mg/kg of talsupram. However, this dose in combination with all the other pretreatments was found to be unsafe, and so for the experimental study the dose of talsupram was lowered to 15 mg/kg. Sham-operated control animals received identical infusions of the ascorbic acid vehicle.

Postoperative testing

Two weeks after surgery, all animals were retested on the touch-screen training schedule to ensure that they could all respond to a square stimulus presented to the right or left of the center of the screen. They then received the following series of tests.

(1) *Retention of the compound discrimination* they had learned immediately prior to surgery.

(2) *A series of three novel compound discriminations or intradimensional shifts (IDS).* Each discrimination required the animal to learn which of two new exemplars from the previously relevant dimension was positively correlated with reinforcement (Fig. 1*c*).

(3) *A probe test.* Following completion of the third IDS, the preexisting exemplars from the irrelevant dimension were replaced with novel exemplars, while the reinforcement contingencies remained the same (Fig. 1*d*). Once the animals had reattained criterion, they were returned to the original discrimination prior to the probe test.

(4) *An extradimensional shift.* A new compound discrimination in which one of the two new exemplars from the previously irrelevant dimension was positively correlated with reinforcement, thus necessitating a shift of an attentional set from one dimension to another (Fig. 1*e*).

(5) *A series of six reversals.* For each reversal the exemplar that had been previously negatively correlated with reinforcement became positively correlated with reinforcement and vice versa. The exemplars from the irrelevant dimension remained uncorrelated with reinforcement (Fig. 1*f*).

(6) *A spatial discrimination and series of four reversals.* Two white squares were presented to the left and right of the center of the screen. For half the animals in the sham-operated group and half the animals in the lesioned group a response to the square on the left was reinforced while for the remaining animals a response to the square on the right was reinforced. Having attained criterion, for each reversal the side that was previously negatively correlated with reinforcement became positively correlated with reinforcement and vice-versa.

(7) *Locomotor activity.* All monkeys were given three daily 1 hr sessions in the activity cage, preoperatively and a 1 hr session during weeks 3, 5 and 7, postoperatively.

(8) *Spatial delayed response.* All monkeys were trained on the spatial delayed response test between 15 and 18 months postsurgery just prior to the termination of the experiment. The experimenter sat directly in front of the subject and at the start of each trial placed a small piece of apple into the opening of one of the test boxes, making sure the monkey was watching. A transparent screen in front of the opening prevented the monkey from attempting to reach out and grab the apple at this stage. Initially, the open side of the box was left facing the monkey and as soon as the transparent screen was removed the monkey could retrieve the apple from inside the box. As training developed, the test box was rotated by 90°, through 135°, until eventually the open side of the test box faced the experimenter and the monkey could only retrieve the apple by reaching through the opening and rotating the box to expose the open side. Once all monkeys were proficient at removing apple from both boxes the test phase began.

At the "sample" stage of each trial the monkey watched as the experimenter placed the apple in one of the two test boxes. An opaque screen was then placed in front of the window and in the "0" sec condition, the opaque and transparent screen was immediately raised and the animal was allowed to make his choice. If he chose the correct box he retrieved the apple, while if he chose the incorrect box he did not. As soon as the monkey had made his choice, both the opaque and transparent screen were lowered and after a 3 sec intertrial interval the opaque screen was raised and a new trial was begun. Monkeys were maintained at the 0 sec delay condition (stage 1) until they chose the correct box on 9 out of 10 consecutive trials. The delay was then increased from 1 sec (stage 2), to 3 sec (stage 3) and then to 6 sec (stage 4) with the same criterion of 9 out of 10 trials correct before the next delay was introduced. As it was possible for the monkeys to use a mediating response during the delay, a final condition (stage 5) was introduced in which during the 6 sec delay period, the monkey was distracted by presenting an extra piece of apple through one of the air holes at the back of the transport cage. In order to retrieve the apple the monkey had to turn away from the window and move to the back of the cage, thus preventing the use of a mediating response. The same criterion as in previous stages was used in this final stage.

Behavioral measures

For the *attentional set-shifting paradigm,* the following behavior measures were used: (1) total number of errors to reach criterion on any one discrimination; (2) the spatial position of the response, that is, right or left; (3) spatial strategy—this was calculated as the number of sessions during which a monkey responded to one side of the screen significantly more than to the other side, expressed as a percentage of the total number of sessions to reach criterion; (4) latency (to the nearest 0.01 sec) to respond to one of the stimuli following their presentation on the VDU (response latency); (5) latency (to the nearest 0.01 sec) to lick the drinking spout following a response to the positive stimulus (lick latency).

For the *spatial delayed response task,* the behavioral measure was total number of errors to reach criterion on any one stage.

In vivo *measurement of dopamine using microdialysis*

The dialysis probes were of a concentric design, made of 23 gauge stainless steel tubing coupled to Hospal dialysis membrane (1.5 mm dialysing surface, 200 μm diameter, nominal 5000 MW cutoff). The probes were perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of 0.5 μl/min. The composition of the ACSF was as follows (mM): NaCl, 120; $NaHCO_3$, 27; KCl, 2.5; NaH_2PO_4, 0.6; Na_2HPO_4, 1.27; Na_2SO_4, 1.0; $CaCl_2$, 1.5; pH 7.4. At room temperature the *in vitro* recovery of the probe for dopamine was 12–14%. The dialysis data were not corrected for recovery. Dialysates were collected every 20 min and 10 μl aliquots subsequently analyzed by microbore HPLC with electrochemical detection. The HPLC system consisted of a BAS PM-60 pump connected to a reverse phase column (Spherisorb 3ODS2, 10 cm × 1 mm i.d.). The mobile phase, flowing at 70 μl/min, was composed of 152 mM citric acid, 15 mM sodium acetate, 1 mM octyl sulfate, 0.8 mM EDTA, and 8% methanol, pH 3.6. Separated compounds [dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid, and homovanillic acid] were detected using a BAS LC-4B cell fitted with a glassy carbon electrode held at a potential of +0.7 V relative to an Ag/AgCl reference electrode. The output from the detector was analyzed automatically by a Trio integrator. Identification/quantification of the peaks was achieved by comparison with external standards of known concentration. The identities of the peaks were further confirmed by spiking with internal standards. Under the conditions described, the maximum sensitivity for dopamine was 0.1 pg (S:N ratio 3).

The probe was implanted acutely into the ventromedial caudate nucleus at the stereotaxic coordinates AP +12.0, L ±2.5, D +10.8. After a 3 hr postimplantation equilibriation period, three baseline determinations were taken, followed by two depolarizing stimuli (75 mM potassium/min over 3 min) separated by three further baseline determinations. Throughout the 7.5 hr experiment the monkey was maintained under Saffan anesthesia (Alphaxalone, 0.9% w/v, Alphadolone acetate, 0.3% w/v; Pitman-Moore Ltd.; 0.4 ml, i.m., every 45–60 min) and its core temperature monitored with a rectal probe and maintained at 37°C. Both lesioned and sham-operated monkeys were taken for dialysis approximately 14–18 months postsurgery.

Assessment of lesions

Measurement of monoamines. All marmosets from the lesioned group, seven of the nine marmosets from the control group, and three marmosets that had received a unilateral 6-OHDA lesion of the prefrontal cortex 10 d earlier, were deeply anesthetized with pentobarbitone, and their brains removed and placed on an ice-cooled dissecting plate. A comprehensive range of cortical areas (defined according to the cytoarchitectonic map of Brodmann, 1909) and subcortical areas were dissected (Fig. 2). By peeling the cortex away from the underlying white matter, dorsolateral prefrontal cortex (1) (area B9), supplementary and premotor cortices (2) (areas B6 and B8), and primary motor cortex (3) (area B4) were removed. The anterior part of the brain was then sectioned into four (blocks A–D in Fig. 2). The first knife cut was made immediately behind the orbits and the three others spaced 2.5 cm apart. The orbitofrontal cortex (4) (areas B10, B11, and B12) and medial prefrontal cortex (5) were removed from block A. The anterior cingulate cortex (6), nucleus accumbens (7), anterior putamen (8), antero-ventromedial head of the caudate (9), and antero-dorsolateral head of the caudate (10) were removed from block B. The midcingulate cortex (11), posterior frontal, and anterior parietal cortex (areas B1, B3, and B5) (12), posterior head of the caudate (13), and midputamen (14) were removed from block C. Finally, the posterior cingulate (15) and posterior parietal cortex (16) were removed from block D.

DA, NA, and 5-HT concentrations were measured using high-performance liquid chromatography (HPLC) with electrochemical detection. Tissue samples were homogenized in 0.2 M perchloric acid and centrifuged, and aliquots of the supernatant were injected directly onto a column via an autosampler. Tissue levels of the monoamines in the lesioned group were compared to those in the sham-operated group to assess the extent of the lesion. Tissue levels in the lesioned group were also compared with those from three monkeys that had received unilateral injections of 6-OHDA 10 d earlier, to obtain an estimate of any recovery that may have taken place over the postsurgery period of 18 months.

Histological evaluation. A marmoset that had received a unilateral 6-OHDA lesion of the prefrontal cortex 10 d earlier and two sham-operated monkeys were anesthetized under barbiturate anesthesia and perfused transcardially with isotonic saline followed by 10% formol saline. Their brains were then removed and postfixed overnight in the fixative before transfer to 30% sucrose at 4°C. The brain tissue was sectioned at 40 μm thickness and every third section through the prefrontal cortex and the head of the caudate was mounted and stained with cresyl violet. This tissue was subsequently used to assess the placement of the dialysis probe, in the case of the two sham-operated monkeys, and to evaluate the extent of any nonspecific tissue damage produced by infusions of 6-OHDA into the prefrontal cortex, in the case of the unilateral-lesioned monkey.

Statistical methods

Regional levels of monoamines in control and lesioned animals were compared using Student's *t* test.

The majority of behavioral results were subjected to analysis of variance using the GENSTAT (Rothamsted) statistical package. Whenever the distribution of these variables violated the assumptions made for the analysis of variance an appropriate transformation was employed. Planned comparisons were made using simple main effects. Likelihood ratio analysis of contingency tables was used when analyzing the results from the spatial delayed response test. This analysis is particularly useful with small cell frequencies and the resulting statistic, 2i, is distributed as χ^2 (Robbins, 1977).

Results

Neurochemical assessment of lesions

Cortical DA

Infusions of 6-OHDA into the prefrontal cortex significantly reduced DA throughout this area (see Table 1). Eighteen months postsurgery the greatest and most consistent reductions were seen in the dorsolateral (B9) (77%) and medial regions (MF) (81%), with slightly more variable reductions occurring in orbital regions (B10/11) (56%). In contrast, although there were reductions in adjacent supplementary and premotor (B6 and B8) and anterior cingulate (C1) areas in a few animals, this was extremely variable and was not significant for the group as a whole. While motor cortex (B4), parietal cortex (Fr2,3), and posterior cingulate (C3) were unaffected, there was a small but significant reduction in DA levels in the midcingulate region (C2) (34%). The most likely explanation for this loss is that some of the dopaminergic fibers to this region took up the 6-OHDA as they passed in front of the genu of the corpus callosum before turning caudally to run in the cingulum bundle.

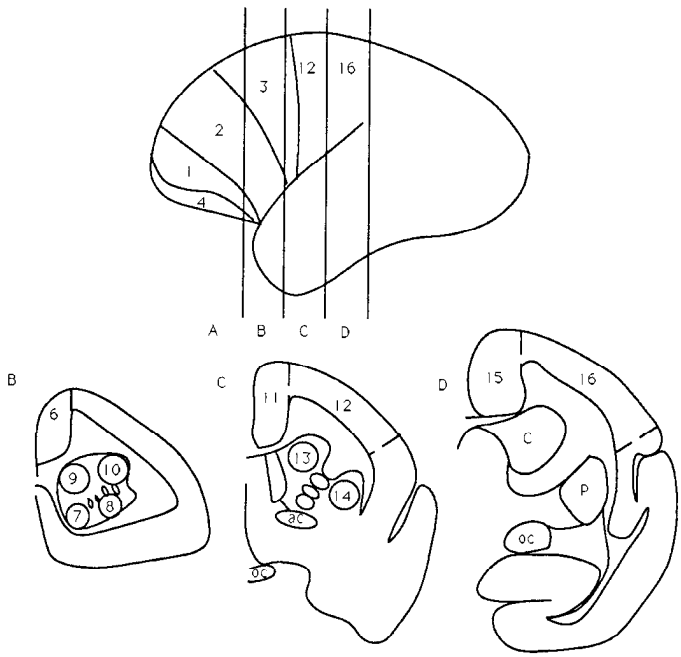


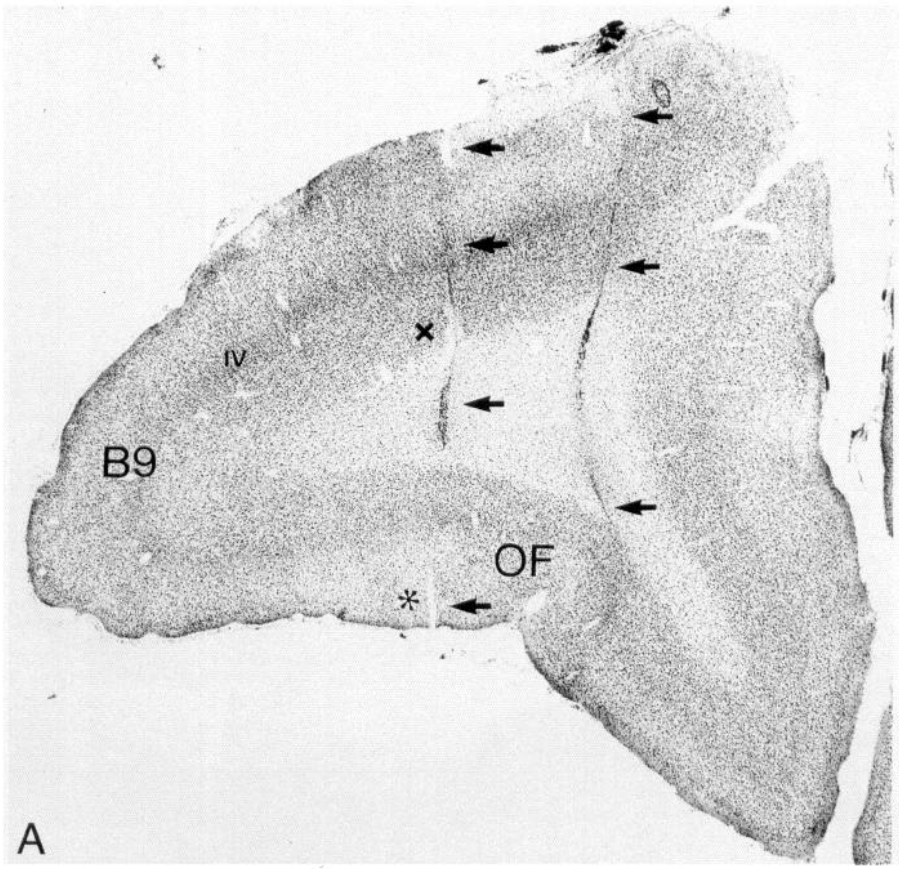
Figure 2. Schematic diagram of the tissue dissection. The outline of the marmoset brain at the *top* illustrates the dissected regions of frontal cortex (*numbers*) along with the position of the knife cuts (*A, B, C,* and *D*). Below are three coronal sections representing the anterior surface of the cut sections *B, C,* and *D*. The *numbered regions within circles* and *within dashed lines* represent the areas taken for catecholamine analysis. *1,* dorsolateral prefrontal cortex; *2,* premotor cortices; *3,* motor cortex; *4,* orbitofrontal cortex; *6,* anterior cingulate cortex; *7,* nucleus accumbens; *8,* anterior putamen; *9,* antero-ventromedial head of the caudate; *10,* antero-dorsolateral head of the caudate; *11,* midcingulate cortex; *12,* posterior frontal and anterior parietal cortex; *13,* posterior head of the caudate; *14,* midputamen; *15,* posterior cingulate; *16,* posterior parietal cortex. *C,* caudate; *P,* putamen; *ac,* anterior commissure; *oc,* optic chiasm.

Table 1. Tissue levels of catecholamines throughout frontal and parietal regions of cerebral cortex in 6-OHDA-lesioned and sham-operated marmosets

	Dopamine			Noradrenaline		
	Control amine levels [$\bar{x}$ (SEM)]	Lesion amine levels [$\bar{x}$ (SEM)]	% reductions [$\bar{x}$ (range)]	Control amine levels [$\bar{x}$ (SEM)]	Lesion amine levels [$\bar{x}$ (SEM)]	% reductions [$\bar{x}$ (range)]
B9	0.052 (0.007)	0.012 (0.003)**	77 (48–100)	0.089 (0.008)	0.030 (0.006)**	67 (35–95)
B10/11	0.071 (0.010)	0.034 (0.004)**	56 (39–88)	0.143 (0.035)	0.067 (0.017)*	58 (16–97)
MF	0.127 (0.040)	0.029 (0.009)**	81 (26–100)	0.162 (0.023)	0.091 (0.026)	58 (12–100)
B6/8	0.062 (0.016)	0.037 (0.008)	46 (4–100)	0.107 (0.030)	0.060 (0.008)	48 (10–78)
B4	0.075 (0.016)	0.067 (0.008)	24 (5–50)	0.151 (0.030)	0.117 (0.009)	25 (18–38)
C1	0.069 (0.012)	0.048 (0.009)	35 (26–100)	0.163 (0.029)	0.192 (0.036)	18 (4–45)
C2	0.073 (0.010)	0.058 (0.012)	34* (15–64)	0.192 (0.021)	0.144 (0.012)*	29 (12–50)
C3	0.058 (0.010)	0.043 (0.004)	23 (10–56)	0.156 (0.027)	0.120 (0.011)	27 (10–69)
Fr2	0.053 (0.006)	0.051 (0.006)	22 (13–40)	0.185 (0.029)	0.129 (0.008)*	24 (9–43)
Fr3	0.051 (0.003)	0.052 (0.007)	15 (4–57)	0.160 (0.021)	0.119 (0.010)	23 (14–38)

Mean levels of DA and NA (expressed as ng/mg wet weight tissue) represent the mean values (±SEM) of 9 control and 11 lesioned marmosets. B9, dorsolateral prefrontal cortex; B10/11, orbitofrontal cortex; MF, medial prefrontal cortex; B6/8, supplementary and premotor cortices; B4, primary motor cortex; C1, anterior cingulate cortex; C2, midcingulate cortex; C3, posterior cingulate cortex; Fr2, posterior frontal and anterior parietal cortex; Fr3, posterior parietal cortex.

*,**, Mean scores of lesioned animals differ significantly from those of the control group at the 5% and 1%, respectively.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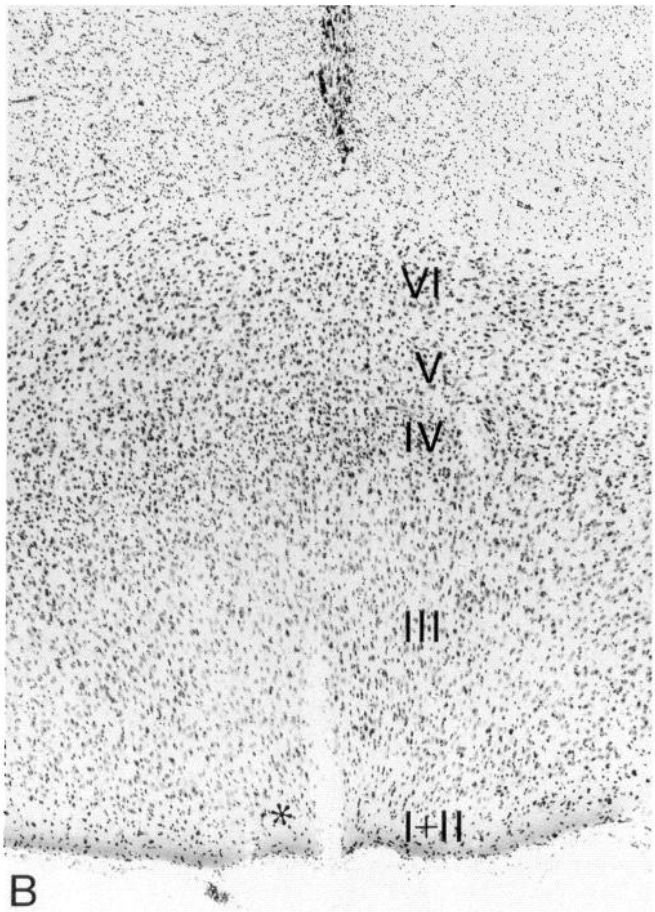
A


VI
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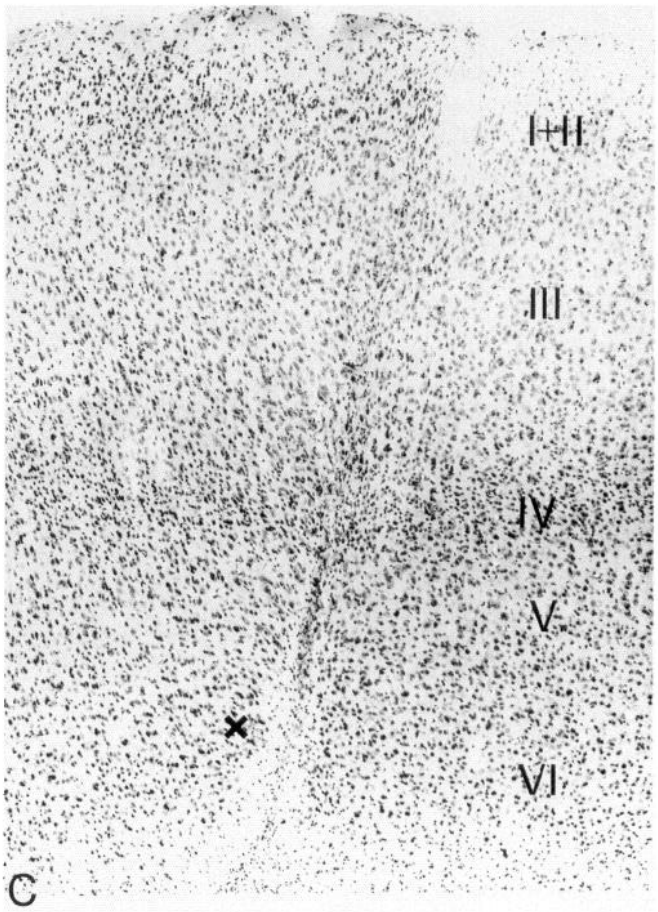
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Cortical DOPAC

In parallel with the reductions in DA levels in the orbital and dosolateral prefrontal regions of lesioned monkeys there were corresponding reductions in the DA metabolite DOPAC [mean DOPAC levels (pg/mg tissue), dorsolateral prefrontal: shams, 0.044 ± 0.008 (n = 5); lesions, 0.018 ± 0.004 (n = 9); orbitofrontal: shams, 0.062 ± 0.018 (n = 4); lesions, 0.045 ± 0.006 (n = 8)]. ANOVA revealed these DOPAC levels to be significantly lower in dorsolateral prefrontal cortex [$F(1,11) = 9.9$, $p < 0.01$] but not in orbitofrontal cortex ($F > 1$). Although DOPAC levels were reduced in the lesioned monkeys, there was an overall increase in the ratio of DOPAC to DA, suggestive of increased turnover, possibly reflecting compensation in the dopaminergic pathway. However, this increase did not reach significance in either dorsolateral [DOPAC:DA ratio: shams, 1.01 ± 0.06 (n = 5); lesions, 3.4 ± 1.2 (n = 9)], or orbitofrontal cortex [DOPAC:DA ratio: shams, 0.96 ± 0.43 (n = 4); lesions, 1.4 ± 0.18 (n = 8)].

Subcortical DA and DOPAC

DA levels in the caudate, putamen, and nucleus accumbens were similar in sham-operated and lesioned monkeys. However, measurement of DOPAC in the head of the caudate revealed a small increase in the ratio of DOPAC to DA in the lesioned monkeys that was significant in the dorsolateral [DOPAC:DA ratio: shams, 0.18 ± 0.11; lesions, 0.29 ± 0.07; $F(1,13) = 11.03$, $p \leq 0.0001$] but not the ventromedial region [DOPAC:DA ratio: shams, 0.23 ± 0.03; lesions, 0.28 ± 0.01; $F(1,13) = 3.91$, $p = 0.068$].

Cortical NA and 5-HT

The noradrenergic uptake blocker talsupram, at a dose of 15 mg/kg, only partially and variably protected the noradrenergic projections to prefrontal cortex from the toxic effects of 6-OHDA. The largest reductions of NA in prefrontal cortex were in the dorsolateral region (67%), with smaller but still significant reductions in the orbital region (58%). Reductions in the medial region were the most variable and were not significant for the group as a whole. Individual comparison of the extent of DA and NA loss throughout prefrontal cortex revealed that in all but two of the lesioned marmosets NA was reduced to a lesser extent than that of DA. Although there were reductions in NA elsewhere in a few animals, these were only significant for the group in midcingulate (29%) and somatosensory cortex (Fr2) (24%).

Pretreatment with citalopram provided full protection of the 5-HT innervation to prefrontal cortex. Without this pretreatment, 6-OHDA injections had been shown to reduce 5-HT by 60–70% (A. C. Roberts, L. S. Wilkinson, B. J. Everitt, and T. W. Robbins, unpublished observations), while in the present study, the greatest mean reduction in 5-HT was 13% in the adjacent premotor areas.

Comparison of these effects of bilateral 6-OHDA injections into the prefrontal cortex at 18 months postsurgery with those of a pilot study using unilateral injections at 3 weeks postsurgery revealed differences in the apparent degree of depletion of DA. Thus, in the pilot study, DA was consistently reduced by >90% in dorsolateral prefrontal cortex, >75% in orbitofrontal cortex, and >85% in medial prefrontal cortex in three unilateral lesioned marmosets. These results may suggest some degree of recovery of presynaptic function in the cortical areas affected by the infusion. However, given that the IDSs, EDS, and first compound reversal had all been performed by the experimental groups within the first 10–12 weeks postsurgery, it would be expected that during this time period the reductions in DA were even greater than the final analysis would suggest.

Histological assessment of lesions

Examination of cresyl violet–stained sections of a bilaterally lesioned marmoset revealed that multiple infusions of 6-OHDA into the prefrontal cortex did not appear to damage intrinsic cell bodies and produced only limited gliosis restricted to the needle tract (Fig. 3). Thus, the reductions in catecholamines observed in this study were a direct result of degeneration of the catecholamine innervation of the prefrontal cortex.

Effects of 6-OHDA infusions into the prefrontal cortex on striatal DA release

The placement of the dialysis probe within the ventromedial region of the caudate nucleus is shown in Figure 4. The levels of DA present in the dialysate samples taken from sham-operated (n = 5) and lesioned marmosets (n = 5) during baseline conditions and following two potassium pulses are presented in Figure 5. Levels of DA during baseline or "resting" conditions were equivalent in both groups of animals throughout the 4 hr collecting period. Following a 3 min pulse of 75 mM potassium, the absolute amount of DA in the dialysate sample of sham-operated monkeys increased approximately twofold within the first 20 min (fourth dialysate sample). Levels then returned to baseline over the following 40 min (fifth and sixth dialysate samples). In contrast, the response in lesioned monkeys was amplified and prolonged, with DA levels increasing fivefold over the first 40 min before returning to baseline over the next 40 min. A similar profile of DA release followed the second potassium pulse with a greater rise in the levels of DA in the dialysate sample of the lesioned monkeys compared to sham-operated controls. In both groups, however, the overall increase was smaller to that seen following the first potassium pulse. ANOVA of the absolute levels of DA across all 12 samples revealed a significant lesion × sample interaction [$F(11,66) = 2.197$, $p = 0.025$]. Further analysis of the simple main effects showed that DA levels in the lesioned animals were higher than sham-operated controls in samples four [$F(11,66) = 10.4$, $p < 0.01$], five [$F(11,66) = 8.1$, $p < 0.01$], and ten [$F(11,66) = 10.48$, $p < 0.01$], which corresponded to the two potassium pulse perfusions.

←

Figure 3. Photomicrograph of a coronal section through the frontal cortex in the marmoset following multiple injections of 6-OHDA into this region 10 d earlier. The path of two needle tracks can be clearly seen extending from the dorsal to the ventral surface (*arrows* in *A*) with a clear line of gliosis along the tracks. When viewed under higher magnification (*B* and *C*), the area immediately surrounding two of the injection sites, marked by * and ×, can be seen not to be associated with a significant degree of cortical neuronal damage. Cortical layers I–VI can be clearly seen. *B9*, Brodmann's area 9; *OF*, orbitofrontal cortex.

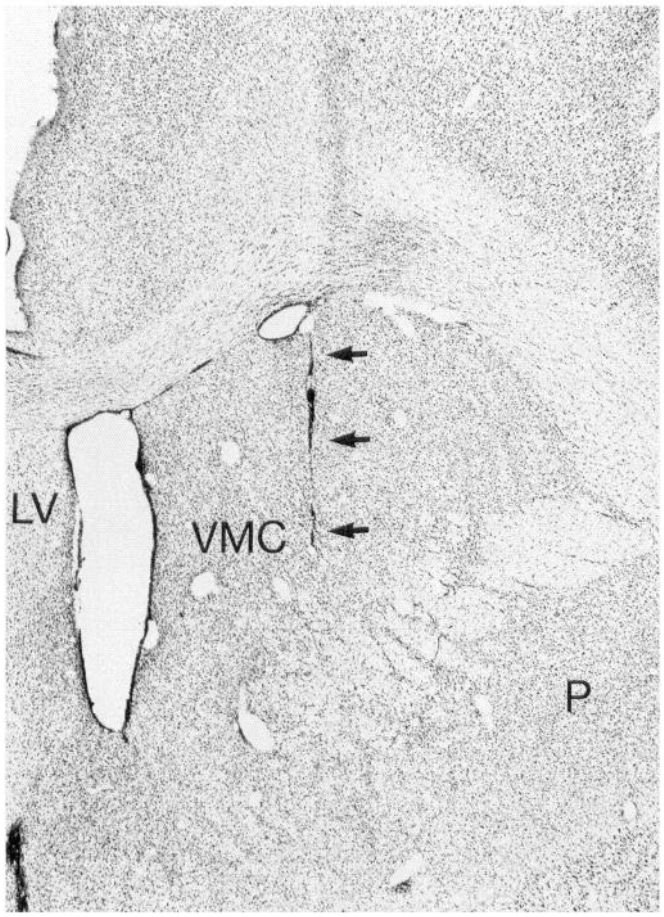

Figure 4. Photomicrograph of the striatum in the marmoset showing the path of the dialysis probe (*arrows*) within the ventromedial caudate nucleus. *LV*, lateral ventricle; *VMC*, ventromedial caudate nucleus; *P*, putamen.

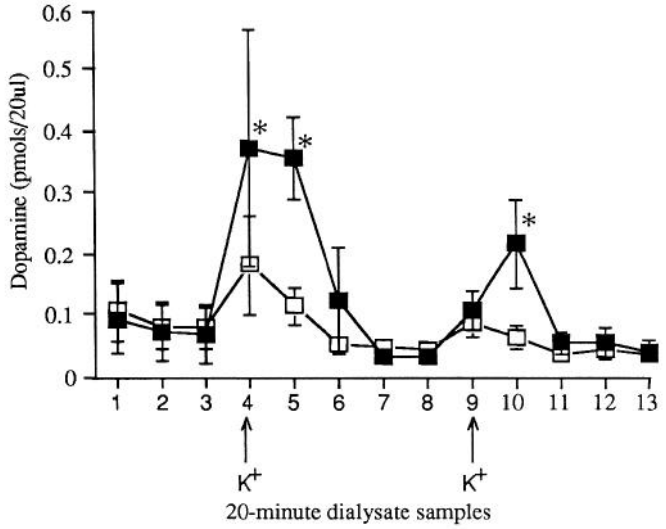

Figure 5. Extracellular concentration of DA in the ventromedial striatum at baseline and following two potassium pulses presented 2 hr apart. At the start of collecting the fourth and ninth dialysate sample, the perfusion medium was switched to one containing 75 mM potassium for 3 min. Values given are the mean concentrations of DA (±SEM) for each successive 20 min dialysate sample, expressed in picomoles. The *open squares* represent sham-operated controls ($n = 5$) and the *solid squares* represent 6-OHDA–lesioned marmosets ($n = 5$). *, Lesioned group significantly different from control group, $p < 0.02$.

Behavioral effects

Retention and new learning of compound visual discrimination problems

Preoperatively, the monkeys scheduled to receive either 6-OHDA or ascorbic acid vehicle into the prefrontal cortex did not differ in their ability to learn either a simple [mean errors to criterion (±SEM): control, 139.6 ± 34.0; lesion, 111.8 ± 27.0; $F < 1$] or compound discrimination [mean errors to criterion combined for the two novel discriminations (±SEM): control, 375.0 ± 92.7; lesion, 320.7 ± 64.03; $F < 1$]. Postoperatively, the performance of lesioned monkeys was equivalent to that of control monkeys on the retention test. Although neither group returned to criterion on the first test session, both groups made considerably fewer errors in reaching criterion compared with their original preoperative learning scores [$F(1,18) = 27.7$, $p < 0.001$] [mean errors to criterion (±SEM) pre- and postsurgery: control, 152.6 ± 70, 34.6 ± 8.4; lesion, 181.2 ± 63, 52.3 ± 18]. Similarly, control and lesioned monkeys did not differ in their acquisition of the subsequent series of novel discriminations requiring IDS [mean errors to criterion (±SEM) D1: control, 219.3 ± 62.9; lesion, 210.5 ± 65.6; D2: control, 198.3 ± 109; lesion, 162.4 ± 37; D3: control, 180.1 ± 50; lesion, 160.9 ± 33]. For statistical purposes, the data was square root transformed ($F < 1$) (Fig. 6). Moreover, trial-by-trial analysis of each monkey's performance across the three IDSs did not reveal any differences in the strategies used in order to solve the discriminations between the control and lesioned groups. For example, the control group adopted a spatial strategy across the three discriminations on 45%, 41%, and 37% of the trials, respectively, while the score for the lesioned group was 43%, 43%, and 29% of trials.

Probe test

The introduction of novel exemplars of the irrelevant dimension resulted in a small decline in performance in both control and lesioned monkeys, although all animals continued to perform significantly above chance. A comparison of the errors made on the probe test session with those made on the previous test session when all monkeys were performing at the 90% criterion level revealed a main effect of the probe [$F(1,16) = 9.92$, $p < 0.01$], but no effect of the lesion ($F < 1$) and no interaction of the lesion with the probe ($F < 1$) (see Fig. 6).

Extradimensional shift

Nearly all monkeys in the control group took considerably longer to acquire the discrimination requiring an EDS compared to the immediately preceding discrimination requiring an IDS (Fig. 7). In marked contrast, only 5 of the 11 monkeys in the lesioned group showed inferior performance on the EDS. Mean errors (±SEM) for the sham-operated group were 346.4 (137) and for the lesioned group, 153.8 (65). ANOVA of square root–transformed errors to reach criterion on the EDS and the preceding IDS revealed no significance of the lesion but a significant lesion × shift interaction [$F(1,16) = 9.13$, $p < 0.01$]. Further analysis of the simple main effects showed that, whereas the performance of monkeys in the control group was significantly inferior on the EDS compared to that of the IDS [$F(1,16) = 6.61$, $p = 0.02$] the monkeys in the lesioned group exhibited

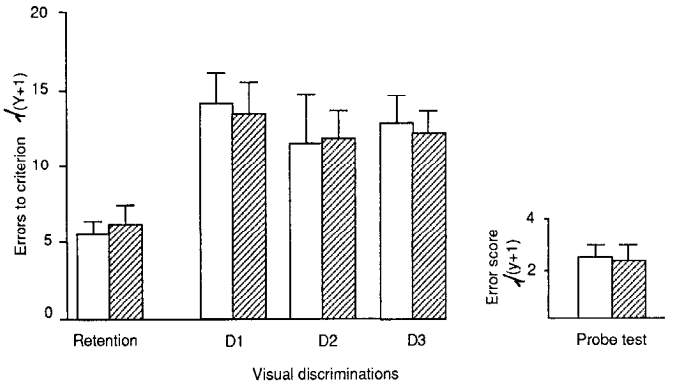

Figure 6. The graph on the *left* shows the mean number of errors made by sham-operated (*open bars*) ($n = 9$) and 6-OHDA–lesioned marmosets (*hatched bars*) ($n = 11$) on the retention test and series of IDSs. The graph on the *right* shows the difference in the errors made during the probe test compared to those made during the preceding session for both groups. Values shown are square roots of the means (±SEM). The lesioned group did not differ from the control group on any of the discrimination tests.

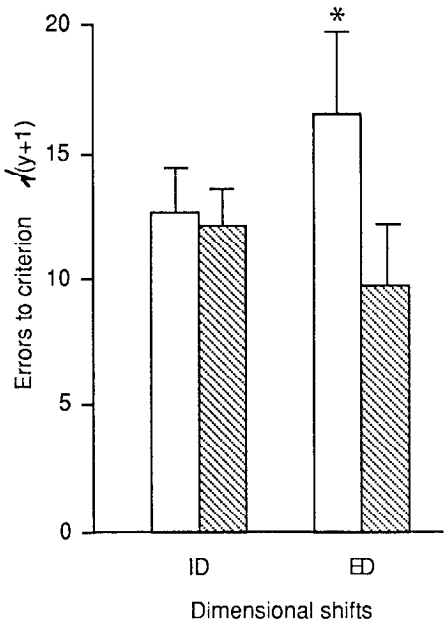

Figure 7. Mean number of errors made by sham-operated (*open bars*) ($n = 9$) and 6-OHDA–lesioned marmosets (*hatched bars*) ($n = 11$) on the final IDS and subsequent EDS. Values shown are square roots of the means ± SEM. *, Significant difference between the lesioned and control group at the 2% level.

comparable performance on both types of shift ($F < 1$). Importantly, although the performance of lesioned monkeys was superior to that of controls at the EDS stage [$F(1,16) = 6.33$, $p = 0.02$], their performance remained equivalent to controls at the preceding IDS stage ($F < 1$).

In order to compare the two groups' performance on the IDS and EDS in more detail, a trial-by-trial analysis of responding was undertaken. One strategy used by the majority of monkeys in a previous study (Roberts et al., 1992) was to respond repeatedly to an exemplar from the previously relevant dimension during the early trials of the EDS; that is, if an animal had been trained on "shapes" it chose to respond to one of the new shape exemplars at the beginning of the discrimination even though one of the line exemplars was the correct stimulus. Indeed, the extent of this perseveration was shown to correlate positively with their overall poorer performance on the EDS (Roberts et al., 1992). Although far fewer animals exhibited this particular strategy in the present study, there were five monkeys from each group that did. Moreover, of the five lesioned monkeys, two of them were still no slower to learn the EDS compared to the previous IDS. This latter finding provides evidence that these lesioned monkeys were still maintaining an attentional set of the previously relevant dimension at the time of the shift, even though they failed to show poorer EDS performance overall.

To determine whether the lesioned monkeys' attentional set-shifting performance was correlated with the percentage depletions of either DA or NA in frontal regions of cortex, including dorsolateral, medial, and orbital prefrontal cortex, premotor and anterior cingulate cortex, the data were subjected to correlational analysis using Pearson's product moment correlation coefficient r. No significant correlations were found between cortical depletions and set-shifting performance, the latter measured either as the total number of errors on the EDS or as the percentage change in performance between the IDS and EDS (r values ranging from -0.29 to 0.543; $p > 0.1$). There were also no significant correlations between attentional set-shifting performance and changes in the ratio of DOPAC to DA in either dorsolateral or orbital prefrontal cortex (r values ranging from -0.27 to 0.40; $p > 0.2$).

Serial discrimination reversals

Over the subsequent series of reversals both lesioned and sham-operated control monkeys showed marked perseveration, continuing to respond to the previously correct exemplar for many trials. However, the extent of this perseveration did not differ between the groups. Further, both the overall level of reversal performance and the improvement in performance across reversals were equivalent in the two groups (Table 2). ANOVA of log transformed data revealed a main effect of reversal [$F(5,90) = 5.50$, $p < 0.01$], but no main effect of the lesion or any interaction of the lesion with the number of reversals or with the type of error made, that is, perseverative or nonperseverative, within a reversal (F values < 1).

Spatial discrimination and reversals

The groups differed in their ability to perform the spatial discrimination. Whereas the mean errors to criterion for the sham-operated controls was 6.3 ± 2.2, that of the lesioned monkeys

Table 2. Mean number of errors made by sham-operated ($n = 9$) and 6-OHDA-lesioned marmosets ($n = 11$) on the series of compound visual discrimination reversals

	Serial compound discrimination reversals					
Group	R1	R2	R3	R4	R5	R6
Control						
$\bar{x}$	2.7	2.7	2.7	2.3	2.2	2.2
(±SEM)	(0.14)	(0.15)	(0.15)	(0.16)	(0.11)	(0.12)
Lesioned						
$\bar{x}$	2.4	2.4	2.4	2.3	2.2	2.2
(±SEM)	(0.13)	(0.14)	(0.11)	(0.08)	(0.08)	(0.11)

Values shown are means of the log transformed data. The lesioned group did not differ from the control group at any stage of reversal learning.

was 21.6 ± 7.7. A one-way ANOVA revealed a significant difference between these scores [$F(1,18) = 5.1$, $p < 0.05$]. The groups did not differ, however, over the following series of reversals ($F < 1$). All monkeys made far fewer errors while performing the series of spatial discrimination reversals as compared to the preceding compound visual discrimination reversals.

Locomotor activity

Activity measures were variable in all marmosets both pre- and postsurgery. However, there were no differences between the lesioned and control groups either within a session or across sessions. ANOVA revealed no effects of the lesion ($F < 1$) or session ($F < 1$) and no interaction between lesion and session ($F < 1$).

Spatial delayed response task

While 10 out of 12 sham-operated monkeys completed all stages of the spatial delayed response task within 400 trials, only 4 of the 10 lesioned monkeys were successful. Likelihood ratio analysis of this contingency table for success and failure confirmed that the lesioned group was significantly inferior to the control group ($2i = 4.57$, $df = 1$, $p < 0.05$). Of the monkeys that did not complete the task, one lesioned monkey failed to reach criterion at the 6 sec delay condition while the rest failed in the distractor condition when the reinforcer was delivered at the rear of the apparatus during the interval between the sample and choice phases.

Although a small number of lesioned monkeys succeeded on the spatial delayed response task, there was no correlation between success and failure on this task and depletions of either DA or NA in any of the regions of frontal cortex examined, as shown by correlational analysis using Pearson's product moment correlation coefficient r (r values ranging from -0.08 to $+0.26$; $p > 0.1$). Moreover, there was no correlation between the lesioned monkeys' attentional set-shifting performance and their success or failure on the delayed response task ($r = -0.031$).

Discussion

6-OHDA lesions of the prefrontal cortex produced profound catecholamine depletion in medial, dorsolateral, and orbital regions and led to improved acquisition of a visual compound discrimination requiring an attentional set shift from one dimension to another (EDS), for example, shapes to lines. In contrast, there was no such effect on acquisition of a discrimination that required the continued maintenance of an attentional set for one particular dimension (IDS), for example, shapes to shapes. Nor was there any effect on a series of visual or spatial discrimination reversals that involved the repeated shifting of responding between two exemplars of the same dimension. In agreement with other published results, however, 6-OHDA lesions of the prefrontal cortex did disrupt performance on a spatial delayed response test.

Behavioral mechanisms

Traditionally, intradimensional and extradimensional transfer tests have been used to study "attentional set" in experimental animals and man (Slamecka, 1968). Superior performance on a discrimination requiring an IDS compared with one requiring an EDS provides evidence for an attentional set (Eimas, 1966; Durlach and Mackintosh, 1986). In the present study, the extradimensional transfer test was used to examine attentional set-shifting ability in monkeys, an ability commonly studied in humans using the WCST. As predicted from our previous findings (Roberts et al., 1988), the control group exhibited superior IDS performance over their EDS performance, confirming that marmosets, like humans, are able to develop an attentional set of the dimensional attributes of compound visual stimuli and are able to switch attentional set from one dimension to another. In contrast, the lesioned group's performance on a discrimination requiring an IDS or an EDS was equivalent and resulted in their performance on the EDS being superior to the control group.

One interpretation of this finding is that the performance of the lesioned group was not governed by an attentional set throughout the series of IDSs and, consequently, the lesioned group did not have to shift an attentional set at the EDS stage. For example, they may have been solving each discrimination on the basis of gestalt or configural images (combining line and shape exemplars into a compound image; see Roberts et al., 1988). Evidence against this interpretation was provided by the resilience of the discrimination performance of the lesioned monkeys to the potential disruption of introducing novel exemplars from the irrelevant dimension during the probe test. Although both groups showed a slight decrement in performance on the probe test, their performance was equivalent and remained significantly above chance.

An alternative explanation for the improved performance of the lesioned monkeys is that they may have solved each discrimination on the basis of specific exemplar knowledge, that is, by learning, for each discrimination, which of the four possible exemplars (two shapes and two lines) was positively associated with reinforcement. Had this been the case, then their performance on the series of IDSs would have been expected to be impaired relative to the control group as, unlike the controls, they would not have had the advantage of having an attentional set for the relevant dimension. Moreover, in the initial stages of the EDS, as many lesioned monkeys as controls responded repeatedly to an exemplar from the previously relevant dimension, providing further evidence that their responding was under dimensional control. Consequently, these results fail to support an account of the behavioral effects in terms of a deficit in attentional control. Instead they favor the hypothesis that the lesioned monkeys were maintaining an attentional set as well as the control group during the series of IDSs but that they were superior at shifting their attentional set from one dimension to another during the EDS.

There are two stages involved in attentional set shifting. First, the monkeys are required to shift their attentional set away from the previously relevant dimension and then they have to refocus their attentional set on the previously irrelevant dimension. However, having learned that a particular dimension is irrelevant, subsequent learning about that dimension may be retarded. In studies of associative learning in animals, retarded learning about previously irrelevant stimuli is referred to as "learned irrelevance" (Baker and Mackintosh, 1977). In the present study, an improvement in attentional set shifting may have been the result, either of a reduction in perseverative responding to the previously relevant dimension, or of faster refocusing of attentional set to the previously irrelevant dimension, perhaps as a result of an impairment in "learned irrelevance." The present study was not designed to differentiate between these two alternatives, although such a distinction has recently been used to differentiate impaired attentional set-shifting performance of

patients with frontal lobe damage and patients with PD (Owen et al., 1993).

The equivalent level of performance of the lesion and control groups on the subsequent series of visual compound reversals and spatial reversals emphasizes the specificity of the behavioral effect. Shifting responding between exemplars within the same dimension would appear not to depend upon the same behavioral and neural mechanisms as shifting "attentional set" between dimensions. 6-OHDA lesions of the prefrontal cortex altered attentional set-shifting ability but had no effect on reversal learning. In contrast, the inverse pattern of effects has been seen following a loss of the cholinergic input to the prefrontal cortex. While prefrontal cortical cholinergic depletion has no effect on attentional set-shifting ability (Roberts et al., 1992) it can disrupt discrimination learning and, in particular, reversal learning (Roberts et al., 1990, 1992). Thus, whereas attentional set shifting is sensitive to manipulations of the dopaminergic system but not the cholinergic system, the reverse is true for discrimination reversal learning. This double dissociation of deficits illustrates the differential contributions made by the cholinergic and dopaminergic forebrain projections to the functions of the prefrontal cortex.

In contrast to the improved ability to shift an attentional set following 6-OHDA lesions of the prefrontal cortex, performance was disrupted on the spatial delayed response task. This is in full agreement with the findings of Brozoski et al. (1979), which demonstrated that an intact prefrontal DA projection was critical for successful performance on this task. In the present study, the deficit was present as long as 18 months postsurgery. The lesioned monkeys succeeded in performing the task as long as they were able to use a mediating response during the delay. When this was prevented, 6 of the 10 lesioned monkeys failed to reach criterion in comparison to only 2 of the 12 sham-operated monkeys.

These differential effects of 6-OHDA lesions of the prefrontal cortex on the two tests of prefrontal function studied here, attentional set shifting and spatial-delayed response, merit careful consideration. The apparently different effects could have a common behavioral basis. For example, an impairment in learned irrelevance, proposed above as one explanation of the superior shifting of "attentional set" shown by the lesioned group, could increase distractibility to task-irrelevant cues, thereby also disrupting delayed-response performance. However, the lack of disruption in the lesioned group upon the introduction of irrelevant stimuli in the probe test of the attentional set-shifting task argues against a simple hypothesis of increased distractibility. Alternatively, an impairment in visuospatial memory, possibly underlying both impaired delayed response performance and impaired spatial discrimination, could have resulted in a fortuitous improvement in EDS performance. While control monkeys inappropriately adopted a spatial strategy early on in the learning process of the EDS stage, the lesioned monkeys may have failed to utilize this maladaptive strategy, due to their impaired spatial function. However, given that the lesioned monkeys adopted a spatial strategy as frequently as controls during the previous series of IDSs, this hypothesis cannot explain their superior EDS performance.

We are left with the conclusion that altered performance on the two tests probably does not have a common behavioral basis and may instead depend upon effects on independent cognitive processes, mediated by different neural systems that are differentially sensitive to depletions of prefrontal catecholamines. Certainly, this latter interpretation is consistent with the lack of correlation between attentional set shifting and delayed response performance in the lesioned monkeys.

Neuroanatomical and neurochemical considerations

Injections of 6-OHDA into the prefrontal cortex significantly depleted DA in medial, dorsolateral, and orbital regions. Just 3 weeks after surgery these depletions had been shown in a pilot experiment to be in the range of 75–95%, and in the present study, were still reduced by between 56% and 81% after 18 months. The reductions of DA in prefrontal cortex were accompanied by smaller, but nevertheless significant reductions in NA that were restricted to dorsolateral and orbital regions. In contrast, pretreatment with citalopram completely prevented any reductions in the levels of 5-HT. Although monoamine levels were within the normal range in adjacent cortical areas, small reductions in DA and NA did occur more distally in the midcingulate region along with a small reduction of NA in somatosensory cortex. However, given that these reductions were in the range of 20–30%, it is unlikely that they had any significant effect. Levels of the DA metabolite DOPAC were also reduced significantly in prefrontal cortex in parallel with the reductions in DA and consequently DOPAC:DA ratios showed no significant increase.

Catecholamine depletion from the prefrontal cortex was accompanied *in vivo* by an increase in extracellular DA in the ventromedial region of the caudate nucleus, as measured by microdialysis using an acutely implanted probe. Although concentrations of DA were similar in the two groups during resting conditions, dopamine release was potentiated in the lesioned group following potassium stimulation. This finding is in agreement with reports in the rat suggesting that reductions of DA in the prefrontal cortex can lead to an upregulation of DA activity in the striatum (Pycock et al., 1980; Glowinski et al., 1988; Louilot et al., 1989; Rosin et al., 1992). However, it is perhaps the first demonstration of such an interaction between cortical and subcortical DA systems in primates. Detection of this interaction so long after the 6-OHDA lesion (14–18 months) confirms that these systems are subject to long-term adaptive changes. Moreover, while the majority of studies have reported alterations in subcortical dopaminergic activity in the nucleus accumbens, only a few (Pycock et al., 1980; Jaskiw et al., 1991), including the present study, have suggested a similar upregulation of neostriatal DA. It is unlikely that the sampling region in the present study included the shell of the nucleus accumbens as the sampling area of a dialysis probe has been shown to extend into the surrounding tissue by no more than 1 mm in rat brain (Ruggeri et al., 1990) and the ventromedial caudate placement was several millimeters from the region of the nucleus accumbens in the marmoset.

Recently, Herve et al. (1991) have suggested that upregulation of striatal DA activity and associated behavioral changes induced by reductions in cortical DA in the rat is blocked by an accompanying loss of cortical NA. However, in the present study, increased dopaminergic activity and behavioral change occurred in the presence of noradrenergic loss, suggesting that either the extent of cortical noradrenergic loss in the marmosets was insufficient to prevent the effects of prefrontal DA loss or the neural control mechanisms are different in primates. Indeed, very little is known about the mechanism underlying the interaction between the cortical and subcortical DA systems, although there is evidence to suggest a direct involvement of

cortical glutamate projections in regulating striatal DA release both at the presynaptic terminal (Keefe et al., 1992) and at the cell body (Kalivas et al., 1989).

Neurochemical basis of the behavioral changes following 6-OHDA lesions of the prefrontal cortex

While the enhanced attentional set shifting in the lesioned monkeys most likely reflects reduced DA in this structure, several alternative possibilities require discussion. For example, it is conceivable that the enhanced performance might result from functional compensation of the prefrontal DA system, particularly in monkeys with partial dopaminergic lesions. However, no correlations were found between behavioral performance and concentrations of DA, the DA metabolite DOPAC, or the DOPAC:DA ratio in the prefrontal cortex. Another possibility is that the NE depletion was responsible, particularly as converging neurobiological and behavioral evidence suggest a role for the ceruleocortical noradrenergic system in processes of selective attention and arousal (Everitt et al., 1992). Thus, reduced prefrontal noradrenergic activity might be predicted to impair IDS performance because it requires selective attention, while enhancing EDS performance by broadening attentional focus. Although there have been a few studies attempting to investigate the effect of central NA function on attentional set shifting in the rat (Mason and Lin, 1980; Pisa and Fibiger, 1983; Devauges and Sara, 1990), the procedures adopted are not directly comparable to those used here, and in any case have led to conflicting results. However, as discussed in the section on "behavioral mechanisms," the profile of behavioral changes in the lesioned marmosets in the present study is not compatible with global impairments in selective attention. However, a loss of prefrontal NA cannot be excluded from contributing to the monkeys' failure to complete the delayed response task. Originally, Brozoski et al. (1979) demonstrated that an 85% loss of NA from prefrontal cortex did not significantly impair spatial delayed response performance in young rhesus monkeys. More recently, however, a number of reports have suggested an involvement of NA in delayed response performance in young (Arnsten and Goldman-Rakic, 1985), aged (Arnsten and Contant, 1992), and MPTP-treated monkeys (Schneider and Kovelowski, 1990).

Reduction in prefrontal DA is also likely to contribute to the delayed response deficits seen in the present study. 6-OHDA lesions of the prefrontal cortex, leading to 87% prefrontal DA depletion, have been shown to produce large deficits in delayed response performance that are reversed by treatment with apomorphine or L-dopa (Brozoski et al., 1979). Studies using a delayed saccade task have also shown these deficits to occur following infusions of a D_1 receptor antagonist into the sulcus principalis (Sawaguchi and Goldman-Rakic, 1991). Thus, if prefrontal DA loss is responsible for the delayed response deficits seen here, it may also underlie the improvement in attentional set shifting, although it is difficult at first sight to understand the basis of such an effect.

Neural basis of attentional set shifting

Frontal lobe damage in man has been shown to impair EDS performance using almost identical tests to those employed here (Owen et al., 1991). More importantly, a similar impairment in EDS performance is also seen in PD (Downes et al., 1989), which is often associated with a loss of prefrontal DA. However, recently, performance on the WCST of a group of PD patients was shown to be negatively correlated with ^{6}L-(^{18}F)-fluorodopa uptake in the medial frontal cortex (Leenders, 1993). Thus, lower tracer uptake was associated with better shifting performance, arguing against a role of prefrontal DA deficiency in set-shifting, in agreement with the present results. There is, of course, a much greater loss of striatal DA in PD, and it is possible that this contributes to the impaired EDS performance, particularly as the DA receptor antagonist haloperidol has also been shown to impair attentional set shifting in humans (Berger et al., 1989). Thus, a plausible hypothesis is that attentional set shifting is mediated by a balanced interaction of prefrontal and striatal DA activity, with enhanced shifting following depressed prefrontal DA function and elevated striatal DA function, and impaired shifting resulting from elevated prefrontal DA function and depressed striatal DA function.

This hypothesis also has the merit of being consistent with a presumed inhibitory role of DA in the prefrontal cortex. Goldman-Rakic and co-workers have shown that the majority of DA receptors form symmetric, presumed inhibitory, synapses on the spines of pyramidal neurons that receive an unspecified excitatory input (Goldman-Rakic, 1992). This synaptic triad occurs mainly in layers V and VI, suggesting that DA can modify the excitability of prefrontal projection neurons to the thalamus and the basal ganglia. The projection neurons to the basal ganglia, in turn, may modify the presynaptic release of striatal DA (Keefe et al., 1992).

Although there is considerable evidence for the existence of corticostriatal functional "loops" with precise connections between different prefrontal regions and different portions of the striatum (Alexander et al., 1986), which of these loops is responsible for attentional set shifting is unclear. Deficits in delayed response performance almost certainly depend on dorsolateral regions of prefrontal cortex (Mishkin, 1957; Gross and Weiskrantz, 1962), but it is controversial whether damage to this region also underlies the impairment on the WCST, although evidence from functional imaging supports this view (Berman and Weinberger, 1990). Studies in monkeys emphasize the importance of orbitofrontal cortex in "set shifting" (Mishkin, 1964; Jones and Mishkin, 1972), but this conclusion has depended on evidence of impaired reversal learning of stimulus–reinforcer associations, which we have shown to be independent of attentional set shifting.

In the present study, extracellular DA was only measured in the caudate nucleus, but as mentioned earlier, upregulation of DA activity following prefrontal DA depletion has also been reported in the nucleus accumbens. Therefore, DA acting within any of the frontostriatal loops could be responsible for the improvement in shifting an attentional set. A number of recent studies, including Gerfen (1992) and Williams and Millar (1990), have provided evidence for a dual action of DA in the striatum that would result in the selective facilitation of some outputs and selective inhibition of others. Such an action might provide a mechanism by which an increase in striatal DA could facilitate attentional set shifting. Indeed, this role in shifting cognitive set may parallel the proposed function of the striatum and its dopaminergic innervation in motor set (Alexander and Crutcher, 1990; Robbins and Brown, 1990).

Relevance to PD and schizophrenia

Development of the attentional set-shifting paradigm for use both in monkeys and humans enables direct comparison of the behavioral impairments associated with selective neurochemical lesions in monkeys with those found in patients suffering

from a variety of neurological or psychological disorders including PD, schizophrenia, Huntington's disease, multiple systems atrophy, and obsessive compulsive disorder. Such comparisons enable the determination of the causal status of many of the neurochemical changes associated with these disorders. For example, in PD, the reduction in DA activity in the striatum can be accompanied by a reduction of dopaminergic and cholinergic activity in the frontal cortex as well as, to a lesser extent, noradrenergic and serotonergic activity in the frontal cortex. Comparison of the behavioral effects of removing selectively the dopaminergic and cholinergic input to prefrontal cortex in a nonhuman primate to those seen in PD reveals that reductions in dopaminergic rather than cholinergic activity most likely are responsible for the attentional set-shifting impairments seen in PD. Whereas there is a reduction in DA activity in both the striatum and frontal cortex of PD and a corresponding impairment in attentional set-shifting ability (Downes et al., 1989), in the present study, a reduction in prefrontal DA activity and a corresponding enhancement in striatal dopamine activity were associated with an improvement in attentional set-shifting ability. Clearly, these opposing effects on shifting an attentional set and on alterations in the activity of striatal DA make it likely that changes in DA activity within the striatum underlie the attentional changes seen in both patients suffering from PD and experimental primates with prefrontal DA depletions.

PD is not the only disorder in which impairments in prefrontal function have been attributed to altered activity in cortical or subcortical DA systems. In schizophrenia, reduced activity in the prefrontal DA pathway together with increased activity in the striatal DA pathway have been proposed as the basis of many of the impairments in prefrontal function associated with this disorder (Jaskiw and Weinberger, 1992). This explanation, however, seems unlikely to account for the impaired performance of schizophrenics on shifting category in the WCST (Stuss et al., 1981). On the contrary, the results of the present study would suggest attentional set-shifting ability should be improved in schizophrenics. Thus, the impairments on the WCST may be more readily attributable to the global "hypofrontality" found in schizophrenics (Berman and Weinberger, 1990).

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