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Neurochemical Correlates of Sparing from Motor Deficits in Rats Depleted of Striatal Dopamine as Weanlings

ABSTRACT: *The behavioral and neurochemical effects of striatal DA depletions were investigated in rats lesioned as weanlings (Day 27) or as adults (250–300 g). Administration of 6-OHDA into the medial forebrain bundle resulted in comparably large (≥95%) depletions of tissue levels of DA in both age groups. As expected, rats depleted of DA as adults exhibited marked deficits in motoric behavior and body weight regulation that persisted for the 8 days of postsurgical observation. In contrast, rats depleted of DA as weanlings were spared from such deficits, and their behavior closely resembled that of age-matched controls. Microdialysis studies revealed dialysate levels of striatal DA that paralleled these age-dependent behavioral differences. At a time when age-related behavioral differences were still quite pronounced (5–6 days postsurgery), basal DA levels were reduced by 80% of control values in rats lesioned as adults whereas basal DA levels in rats lesioned as weanlings were unchanged relative to their controls. Finally, adults depleted of striatal DA as weanlings were no more sensitive to the movement-impairing effects of intrastriatal sulpiride (3.0 or 10.0 μg/hemisphere) infusions than were control rats. These data suggest that weanlings compensate for large, but incomplete, denervation of striatal DA with markedly enhanced release and turnover from residual terminals. This developmental plasticity may prevent the occurrence of behavioral deficits soon after the lesion and also the supersensitivity to the challenging effects of DA antagonists as animals grow into adulthood.*
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Keywords: *striatum; dopamine; movement; 6-OHDA; adult; weanling; development; recovery of function; microdialysis; sulpiride; Parkinsonism; rat*

Extensive (i.e., $>85\%$) depletions of striatal dopamine (DA) in adults rats, resulting from intracerebral injections of the neurotoxin 6-hydroxydopamine (6-OHDA), lead to a well-characterized syndrome of behavioral deficits that include aphagia, adipsia, akinesia, catalepsy, and somatosensory neglect (for review, see Zigmond & Stricker, 1989). DA-depleted adults partially recover, over the course of several weeks to months, from many of these deficits provided that the depletions are not too large (i.e., $<90\%$). An important neurochemical mediator of this gradual and partial recovery of function is the emergence of several pre- and postsynaptic compensations that occur within residual (nondamaged) dopaminergic neurons and their targets that collectively serve to restore a sufficient level of dopaminergic transmission within the basal ganglia (Zigmond, Abercrombie, Berger, Grace, & Stricker, 1990). The necessary contribution of residual striatal DA receptor activity to the expression of these ''recovered behaviors'' is demonstrated by the observation that the initial deficits can be rapidly reinstated following systemically or locally administered D1 or D2 receptor

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antagonists at doses that are far lower than those required to induce temporary ingestive or sensorimotor impairments in intact rats (Heffner, Zigmond, & Stricker, 1977).

In contrast, comparably large depletions of striatal DA sustained during development result in different behavioral effects, and the degree of departure from the severe impairments outlined earlier is a function of the animal's age at the time of lesion (for a review, see Bruno, Sandstrom, Arnold, & Nelson, 1998). Rats sustaining very large (i.e., $>90\%$) depletions of striatal DA as weanlings [postnatal Day (PD) 27] exhibit very modest ingestive and sensorimotor deficits and recover from deficits in a matter of days as opposed to the weeks to months required for recovery in rats depleted of DA as adults (Weihmuller & Bruno, 1989). Moreover, these behavioral deficits do not reappear as the lesioned animals reach adulthood. The neurochemical basis for this rapid recovery from modest deficits is not completely understood. It appears that DA receptor activation remains a necessary condition for the expression of motor behavior in these animals, as the systemic administration of DA antagonists in adults depleted of DA as weanlings results in significant motoric deficits. Curiously, however, rats depleted of DA as weanlings do not exhibit the *supersensitivity* to the behavioral effects of DA antagonists that is characteristic of rats depleted of DA as adults; instead, they exhibit a sensitivity comparable to that of intact rats (Sandstrom & Bruno, 1997). We have speculated that this unexpected lack of behavioral supersensitivity reflects an increase in the synthesis and release of DA from residual DA terminals to such an extent that extracellular levels of DA no longer differ from those in intact controls. This remarkable compensation was postulated to underlie both the rapid recovery of function and the normal sensitivity to competitive DA antagonists (Bruno et al., 1998).

The present study further evaluated this hypothesis. In Experiment 1, we utilized microdialysis techniques to estimate extracellular levels of striatal DA in awake rats. We compared basal DA efflux in rats treated with 6-OHDA as adults with efflux in rats treated with 6-OHDA as weanlings. Our hypothesis was that 5 to 6 days after the administration of 6-OHDA, when weanlings were no longer exhibiting behavioral deficits, basal DA efflux would be comparable to that seen in vehicle-treated controls. In contrast, we predicted that at this same time postlesion, DA efflux in rats depleted as adults would still be very much reduced relative to control levels. In Experiment 2, we replicated our previous study on the sensitivity of adult rats depleted of DA as weanlings to the behavioral effects of competitive direct DA antagonists. However, in this experiment the DA antagonist was infused directly into the denervated striatum, as opposed to the previous systemic administration (Sandstrom & Bruno, 1997) to specifically assess the necessity of residual striatal DA receptor activity in the expression of motor behavior in adulthood.

GENERAL METHODS

Animals

Male Sprague-Dawley albino rats served as subjects for these experiments and were produced in our breeding colony (weanlings) or purchased as adults (225–250 g; Zivic Miller, Allison Park, PA). Litters bred in our colony were culled to 8 to 10 pups and remained with the dam in hanging plastic tubs ($48 \times 27 \times 20$ cm) until they were weaned on PD 21. Animals purchased as adults were housed in wire cages ($42 \times 25 \times 18$ cm). Rats sustaining DA depletions and requiring special care were initially housed in an incubator maintained at 35°C and 65% humidity until they were able to voluntarily ingest food and maintain body weight. Weights were monitored daily, and supplemental intragastric feedings (STAT nutritional supplement; PRN Pharmacal Inc., Pensacola, FL) were administered as needed to maintain the body weight and hydrational balance of the animals while in the chamber. Food (pellets and soft mash) and water were available ad libitum.

DA Depletions

On the day of surgery, adult animals (250–300 g) were anesthetized with ketamine (85.0 mg/kg, ip) and xylazine (5.0 mg/kg, ip), and weanling animals (PD 27) were anesthetized with only ketamine (85.0 mg/kg, ip). Rats in each experiment were infused with either 6-OHDA or its vehicle solution (1.0 mg/ml ascorbic acid in 0.9% NaCl). Infusions targeted the posterior aspect of the medial forebrain bundle in a location shown to produce large striatal DA depletions following a single administration of 6-OHDA (Ervin, Fink, Young, & Smith, 1977). Coordinates for the infusions in adults were 5.0 mm anterior to and 2.0 mm lateral from the interaural line, 8.0 mm ventral from the dural surface. Coordinates for the infusions in weanlings were 3.2 mm anterior to and 1.7 mm lateral from the interaural line, 8.0 mm ventral from the dural surface. Infusions were made with Hamilton syringes (26-gauge, 10.0 μl, 5.0-cm beveled tips; Hamilton, Reno, NV). All animals received bilateral infusions (4.0 μl) of 6-OHDA (8.0 μg in adults, 10.0 μg in weanlings; dose expressed as free base) or vehicle.

Tests of Motoric Behavior

A battery of tests was used in both experiments to determine the effects of DA depletions on spontaneous motoric behavior (Experiment 1) and the impairing effects of an acutely administered DA antagonist (Experiment 2). An *akinesia* test measured the latency (120-s ceiling) for the animal to move all four limbs and to initiate locomotion when placed into a novel environment ($45 \times 24 \times 21$ cm opaque plastic tub). An *open-field activity* test measured the level of spontaneous exploratory locomotion over a wide area ($1.2 \times 1.2 \times 0.46$ m—divided into 36 squares of 20×20 cm each). Locomotion was quantified by

tracking the number of grid crossings during the first 10 s of 30-s intervals, for a total of 3 min. A *catalepsy* test measured the animals' ability to initiate movement to extricate itself from an abnormal posture. The hind limbs were placed on an elevated platform (adults = 7.0 cm high, weanlings = 3.5 cm high), and the latency required to place the hind paws on the same surface as the forepaws was recorded (120-s ceiling).

Quantification of DA Depletions

At the end of the experiment, rats were sacrificed by decapitation, and their brains were rapidly removed and carefully frozen with powdered dry ice. Brains were stored at −70°C until sectioned for probe/cannula placements (discussed later). The extent of the DA depletions in tissue was determined by measuring DA content in bilateral striatal micropunches (1.0 mm^3) from dorsolateral (ventral to the corpus callosum) and ventromedial (dorsal to the accumbens and just medial to the lateral ventricles). Sites levels of DA in these micropunches were determined using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Micropunches were homogenized via sonication in 100 µl of 0.1 N $HClO_4$ containing 0.2 mM Na-bisulfite and then centrifuged for 20 min at 12,000 rpm. Ten microliters of supernatant was injected into the HPLC-ED system (PM-80 isocratic pump and LC-4C detector; Bioanalytical Systems, Inc., West Lafayette, IN), and separated using a reverse-phase microbore column (Sepstik MF8949, 100 × 1 mm, C-18; BAS, Inc.). The mobile phase consisted of 0.1 M monochloroacetic acid, 0.1 mM EDTA, 1.2 mM sodium octyl sulfate, and 5% acetonitrile (by vol). The working electrode was set at a potential of +550 mV. The centrifuge pellet was analyzed for protein content using a standard protein assay kit (Pierce, Rockford, IL), and DA content was expressed as pmol DA/mg protein.

Verification of Placement of Microdialysis Probe and Infusion Cannula

Frozen brain was sectioned (40 µm, coronal) using a freezing cryostat. Sections were thaw mounted onto gelatin-coated slides and subsequently Nissl stained with cresyl violet to expose astrocytic scarring that surrounds the insertion of dialysis probes and infusion cannula. Only animals with probe and cannula placements within the medial regions of the dorsal striatum were included in this article.

EXPERIMENTAL PROCEDURES

Experiment 1: DA Efflux in Rats Treated With 6-OHDA or Vehicle as Weanlings or as Adults

Immediately following the infusion of 6-OHDA or its vehicle (discussed earlier), rats were implanted with a chronic unilateral guide cannula for subsequent microdialysis testing. The coordinates for the guide cannula for adult animals were 0.5 mm anterior and 2.6 mm lateral from Bregma, 3.6 mm ventral from dura. The coordinates for the weanlings were 0.7 mm anterior and 2.6 mm lateral from Bregma, 4.0 ventral from dura. These coordinates positioned the microdialysis probe into the medial region of the dorsal striatum. A locking dummy stylet that ended flush with the guide cannula was inserted until the day of the microdialysis session.

At either 5 or 6 days following surgery, microdialysis testing was conducted in circular plastic containment systems (48 cm in height, 38 cm in diameter). Concentric microdialysis probes (0.5 mm o.d., 4.0-mm membrane tip for adults, 3.0-mm membrane tip for weanlings; BAS, Inc.) were inserted into the guide cannula and perfused (2.0 µl/min) with artificial cerebrospinal fluid (aCSF) containing (in mM): 155 Na^+, 2.9 K^+, 1.1 Ca^{2+}, 0.83 Mg^{2+}, 132.8 Cl^-, 5.9 glucose, and 0.1 µM of the acetylcholinesterase inhibitor neostigmine. The inclusion of neostigmine was necessary to detect basal levels of striatal ACh using relatively small concentric probes (ACh data are not reported in this article). Awake animals were perfused for an initial 4-hr discard period to permit the equilibration of a stable and maximally TTX-sensitive baseline (Nakamura, Goshima, Yue, & Misu, 1992) prior to the collection of dialysates at 15-min intervals. All dialysis vials contained 10 µl of a 0.05 N $HClO_4$ solution containing sodium bisulfite (0.1 mM) and EDTA (2.0 mM) as an antioxidant (see Robinson & Whishaw, 1988). Collections for DA efflux were taken at alternating 15-min intervals (Collections for ACh were taken during the subsequent 15-min intervals; data not reported in this article.) for a period of 2 hr. DA efflux values were corrected for absolute probe recovery as determined in vitro using a concentration of DA close to expected basal values. Animals were tested on the motor battery starting 2 days prior to surgery and continuing for the 8 days following surgery. A total of 15 weanlings (9 vehicle- and 6 6-OHDA-treated) and 17 adults (10 vehicle- and 7 6-OHDA-treated) were included in this analysis.

The extent of DA depletions was analyzed using a three-way ANOVA with age (adult vs. weanling) and lesion (vehicle vs. 6-OHDA) as between-subject factors and region (dorsal vs. ventral) as a within-subject factor. Data on DA efflux were analyzed using three-way ANOVAs with age, lesion, and time (microdialysis collection interval) as factors. Data on motoric behavior were analyzed using three-way ANOVAs with age, lesion, and days (days postsurgery) as factors. Body weight data were limited to postsurgical changes in weight in rats lesioned as weanlings and adults. Thus, a two-way ANOVA (age, days) was conducted on body weight expressed as a percent of presurgical baselines. Significant two- and three-way ANOVAs were further analyzed by lower order ANOVAs. Post hoc analyses (*t* tests) were conducted following the determination of significant main effects or interactions. In all cases, the number of post hoc tests was limited to only those necessary to reveal the source of significance to reduce the likelihood of Type I errors (Keppel, 1991). In all cases, statistical significance was defined as $\alpha = 0.05$.

Experiment 2: Effects of Intrastrial Infusions of Sulpiride on Motoric Behavior in Rats Treated With 6-OHDA or Its Vehicle as Weanlings or as Adults

Upon reaching adulthood (Days 60–80), animals that received vehicle or 6-OHDA infusions as weanlings (PD 27) were

implanted with bilateral guide cannula for intrastriatal infusion of the D2 antagonist, sulpiride. Rats were anesthetized with ketamine (85.0 mg/kg, ip) and xylazine (5.0 mg/kg, ip), and cannula (Plastics One, Roanoke, VA) were implanted bilaterally (5.0 mm apart) into the medial aspect of the dorsal striatum at the following coordinates (0.7 mm anterior to and 2.5 mm lateral from Bregma, 4.5 mm ventral from dura). Cannula were secured with skull screws and dental cement. Bilateral dummy stylets were inserted to prevent clogging. Animals were allowed to recover for 3 days prior to the onset of the infusions.

On each test day, animals were transported from the colony room to the testing room and allowed to habituate for 30 min prior to testing. The motor test battery (discussed earlier) was performed on all animals prior to the intrastriatal infusion to obtain baseline levels of responding. Infusion cannula (27 gauge) were then inserted into the guides, and animals received sulpiride (3.0, 10.0 μg/hemisphere) or vehicle (0.9% saline) in a volume of 0.5 μl delivered over 1 min. Each dose was tested in every animal, on alternating days in counterbalanced order. Cannula remained in place for 1 min following the infusion to allow for drug diffusion. The cannula were then removed, dummy stylets inserted, and the rats were tested at 15, 30, and 60 min postinfusion.

The extent of DA depletions was analyzed using a two-way ANOVA with lesion (vehicle vs. 6-OHDA) as a between-subject factor and region (dorsal vs. ventral) as a within-subject factor. Data on motoric behavior were analyzed using three-way ANOVAs with lesion, dose (0, 3, 10 μg), and time (baseline, 15, 30, 60 min) as factors. Post hoc analyses (*t* tests) were conducted following the determination of significant main effects or interactions. In all cases, the number of post hoc tests was limited to only those necessary to reveal the source of significance to reduce the likelihood of Type I errors (Keppel, 1991). In all cases, statistical significance was defined as $\alpha = 0.05$.

RESULTS

Experiment 1

DA Depletions. Mean (±*SEM*) levels of DA in striatal micropunches, from rats treated as adults or as weanlings, are expressed in either absolute levels (pmol DA/mg protein) or as a percentage of control values in Table 1. As expected, overall levels of striatal DA were significantly lower in weanlings than in adults, $F(1, 56) = 21.353$, $p < 0.001$. This difference was accounted for by the values in vehicle-treated adults being greater than values in vehicle-treated weanlings ($p < 0.05$). Administration of 6-OHDA resulted in extensive depletions of striatal DA, $F(1, 56) = 223.124$, $p < 0.001$. Inspection of these values reveals that the DA depletions were comparable between the two age groups in both dorsal and ventral regions of striatum ($ps > 0.05$).

Body Weights. Pre- and postoperative body weights ($M \pm SEM$) for the four treatment groups are illustrated in Figure 1. All body weights were normalized as 100% on the day of surgery (presurgery). The body weights of vehicle-treated controls are shown again on Day 8, and they reflect, as expected, the continued growth of the weanlings and the stable weights of the adults. The postsurgical body weight profiles of the two lesioned groups differed significantly, $F(1, 11) = 6.205$, $p < 0.05$. Rats depleted of DA as weanlings continued to grow and seldom required intragastric feedings particularly beyond the second postsurgical day. In contrast, rats depleted of DA as adults did not return to their presurgical baseline despite numerous intragastric feedings each day

Table 1. Striatal Tissue Dopamine Depletions

Condition	Dorsal DA	Ventral DA	% Depletion	
Experiment 1: Microdialysis				
Adult rats				
Vehicle (10)	76.27 ± 6.64	56.89 ± 5.96	Dorsal	Ventral
6-OHDA (7)	1.18 ± 0.47	1.97 ± 1.01	−98.45 ± 0.62	−96.53 ± 1.79
Weanling rats				
Vehicle (9)	32.23 ± 1.80	35.63 ± 3.71		
6-OHDA (6)	0.94 ± 0.31	1.63 ± 0.30	−97.08 ± 0.95	−95.43 ± 0.85
Experiment 2: Intrastriatal sulpiride				
Vehicle (12)	36.83 ± 3.77	40.39 ± 4.02		
6-OHDA (8)	0.66 ± 0.22	5.31 ± 1.99	−98.21 ± 0.61	−86.86 ± 4.94

Mean (±*SEM*) content of DA (pmol/mg protein) in striatal regions for vehicle- and 6-OHDA-treated rats used in Experiment 1 (top) and Experiment 2 (bottom). In both experiments, DA was assayed from dorsal and ventral striatal micropunches. The right-hand column expresses DA depletions as a percent reduction from age-matched, vehicle-treated controls. DA depletions were very large and comparable between the two age groups, the two regions, and the two experiments.

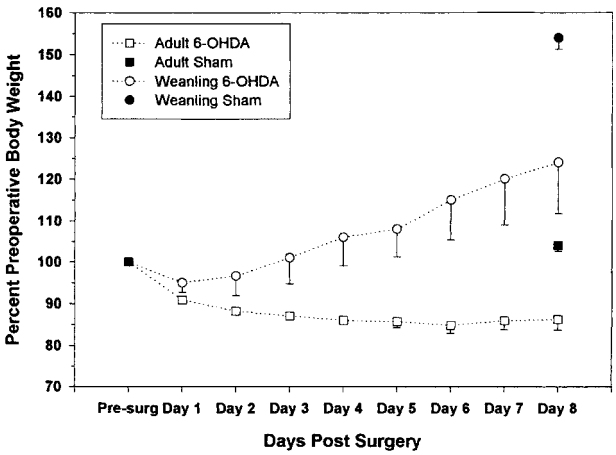

FIGURE 1 Mean ($\pm SEM$) body weights for adult and weanling rats prior to (Pre-surg) and for the 8 days following the infusion of vehicle or 6-OHDA into the medial forebrain bundle. Body weights were normalized as a percent change from the Pre-surg baseline values. Postsurgical body weight data from vehicle-treated controls is shown only for Day 8 to illustrate the relative deviation of the dopamine-depleted rats from age-matched controls. The figure illustrates that while both depleted groups were significantly below age-matched controls, rats depleted as weanlings were progressively gaining weight whereas those depleted as adults were still below their own presurgical baselines. Adult 6-OHDA, $n = 7$; Adult Sham, $n = 10$; Weanling 6-OHDA, $n = 6$; Weanling Sham, $n = 9$.

(days: $F(7, 77) = 4.798$, $p < 0.05$; Days × Age interaction, $F(7, 77) = 8.437$, $p < 0.05$). A restricted number of post hoc comparisons revealed that body weights of the two groups of lesioned rats, expressed as a percentage of age-matched controls, were comparable on Day 1 postsurgery, but differed significantly on Days 4 and 8, $t(11) = -2.492$, $p < 0.05$; $t(11) = -2.676$, $p < 0.05$, respectively.

Motoric Behavior. Pre- and postsurgical motoric behavior as measured by the duration to move all four limbs (akinesia) and the level of open-field activity are represented in Figure 2. In terms of akinesia latencies (top panel), the most relevant overall statistic revealed by the ANOVA was the striking Age × Lesion × Day interaction, $F(8, 216) = 4.092$, $p < 0.001$. The major source of this interaction was revealed by conducting separate ANOVAs for each of the two ages. With regard to the weanlings, there was no significant effect of lesion or day (ps > 0.05). With regard to the adults, 6-OHDA-treated rats exhibited far greater akinesia latencies than their vehicle-treated controls, $F(1, 14) = 61.96$, $p < 0.001$. Post hoc comparisons revealed that these differences were seen on both Day 1, $t(14) = -6.975$, $p < 0.001$, and Day 8, $t(14) = -2.532$, $p < 0.05$.

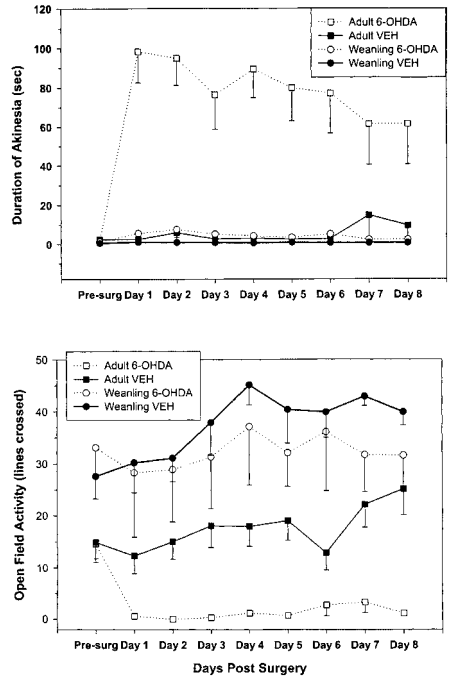

FIGURE 2 Top Panel: Mean ($\pm SEM$) duration of akinesia (s) for adult and weanling rats prior to (Pre-surg) and for the 8 days following the infusion of vehicle or 6-OHDA into the medial forebrain bundle. See the Methods for details of the akinesia test. Rats depleted of DA as adults exhibited marked increases in the duration of akinesia beginning the first day after the lesion that persisted (although to a somewhat smaller extent) throughout the 8-day period. The duration of akinesia in the other three treatment groups did not change from baseline (Pre-surg) values. Bottom Panel: Mean ($\pm SEM$) open-field activity (line crossings) for adult and weanling rats prior to (Pre-surg) and for the 8 days following the infusion of vehicle or 6-OHDA into the medial forebrain bundle. See the Methods for details of the open-field test. Overall, weanling rats were more active than adult rats. Rats depleted of DA as weanlings exhibited a high level of open-field activity, comparable to age-matched controls. In contrast, rats depleted of DA as adults exhibited next to no open-field activity, largely remaining in the initial start position for the duration of the test.

In terms of open-field activity (bottom panel), there was an overall effect of age, $F(1, 27) = 29.70$, $p < 0.001$, that reflected the greater activity of weanlings relative to adults. However, there was not an overall Age × Lesion × Day interaction ($p > 0.5$). Visual inspection of the data suggests age-related differences. As was seen for the measure of akinesia, there were no differences between vehicle- and 6-OHDA-treated weanlings. Rats treated with 6-OHDA as adults exhibited significantly fewer line crossings than their age-matched controls, essentially freezing in the open field for the duration of the test period.

The ability of DA-depleted rats to move out of an abnormal posture was measured by the latency to remove the hind paws from an elevated block (catalepsy); the results are illustrated in Figure 3. As was the case with akinesia, there was a significant overall Age × Lesion × Day interaction, $F(8, 216) = 4.93$, $p < 0.001$. Again, the marked age dependency in catalepsy latencies was revealed with separate ANOVAs for each age. For the weanlings, there were no significant effects of lesion or day ($ps > 0.1$). In contrast, an analysis of latencies from adult rats revealed significant effects of lesion, $F(1, 14) = 70.65$, $p < 0.001$, day, $F(8, 112) = 8.24$, $p < 0.001$, and a Lesion × Day interaction, $F(8, 112) = 4.93$, $p < 0.001$. DA-depleted rats exhibited near-ceiling-level latencies on the first postsurgical day, $t(14) = -7.268$, $p < 0.001$, that were still higher than age-matched controls on postsurgical Day 8, $t(14) = -3.123$, $p < 0.05$.

Microdialysis Probe Placement. Histological examinations revealed that all animals in Experiment 1 had probe placements in the medial aspect of the anterior striatum. There were no systematic differences in the probe placements of animals from the four treatment conditions. A representative photomicrograph from a rat treated with 6-OHDA as a weanling appears as Figure 4A.

Striatal DA Efflux. Mean (±*SEM*) basal DA efflux for each of the four treatment conditions is illustrated in Figure 5. Given that the critical comparisons are between vehicle- and 6-OHDA-treated rats within an age group, separate ANOVAs from weanlings and adults were conducted from the outset. Basal DA efflux was significantly lower in adults treated with 6-OHDA relative to vehicle-treated controls, $F(1, 15) = 5.139$, $p < 0.05$. In contrast, basal efflux in weanlings treated with 6-OHDA was not significantly lower than controls ($p > 0.5$) despite the fact that tissue levels of DA were markedly reduced (95–97%) relative to controls and comparable to the large depletions of tissue levels seen in adults treated with 6-OHDA (see Table 1) Figure 6.

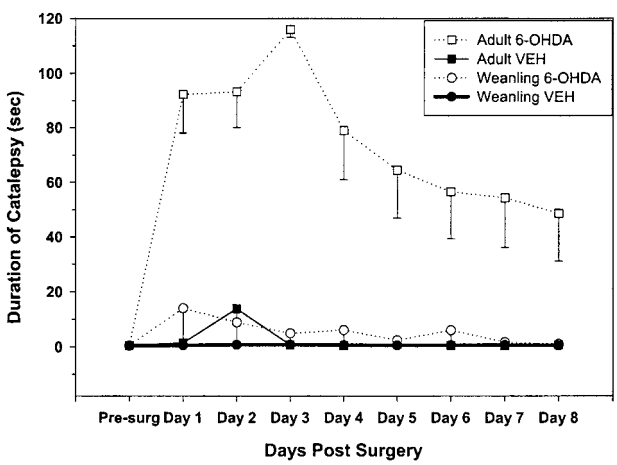

FIGURE 3 Mean (±*SEM*) duration of catalepsy (s) for adult and weanling rats prior to (Pre-surg) and for the 8 days following the infusion of vehicle or 6-OHDA into the medial forebrain bundle. See the Methods for details of the catalepsy test. Rats depleted of DA as adults exhibited marked increases in the duration of catalepsy beginning the first day after the lesion that persisted (although to a somewhat smaller extent) throughout the 8-day period. The duration of catalepsy in the other three treatment groups did not change from baseline (Pre-surg) values.

Experiment 2

The results of Experiment 1 demonstrate a significant neurobehavioral plasticity in rats depleted of DA as weanlings. First, they do not exhibit the pronounced motoric deficits seen in rats comparably depleted of DA as adults. Second, despite the fact that striatal tissue levels were reduced by greater than 95% in depleted weanlings, extracellular values of DA, as estimated by microdialysis, never fell outside of the control levels. This was in marked contrast to the large decline in extracellular DA seen in depleted adults. Experiment 2 was designed to reveal a behavioral consequence of this unexpected maintenance of extracellular DA levels in lesioned weanlings. The experiment determined the sensitivity of adults, depleted of DA as weanlings, to the motor-impairing effects of D2 antagonists. The supersensitivity to the impairing effects of D2 antagonists in rats *depleted of DA as adults* has frequently been cited as a sensitive behavioral assay for significant reductions in striatal DA release (Heffner et al., 1977). We predicted that the maintenance of control-like levels of extracellular DA in rats depleted as weanlings would prevent the behavioral supersensitivity to the effects of local administration of the D2 antagonist, sulpiride.

DA Depletions. The extent of striatal DA depletions for adult rats treated with vehicle or 6-OHDA as weanlings is illustrated in the bottom of Table 1. Again, the DA depletions were quite extensive (i.e., 87 and 98% in ventral and dorsal regions of striatum, respectively) and were comparable to those observed in Experiment 1. An analysis of tissue levels of DA revealed a highly significant effect of lesion, $F(1, 36) = 103.48$, $p < 0.001$, but no effect of striatal region or a Lesion Condition × Region interaction ($ps > 0.05$).

Placements of Intrastriatal Injection Cannula. Histological examination revealed that the bilateral injection cannula in all animals utilized in Experiment 2 terminated in the medial striatum (see Figure 4B for a

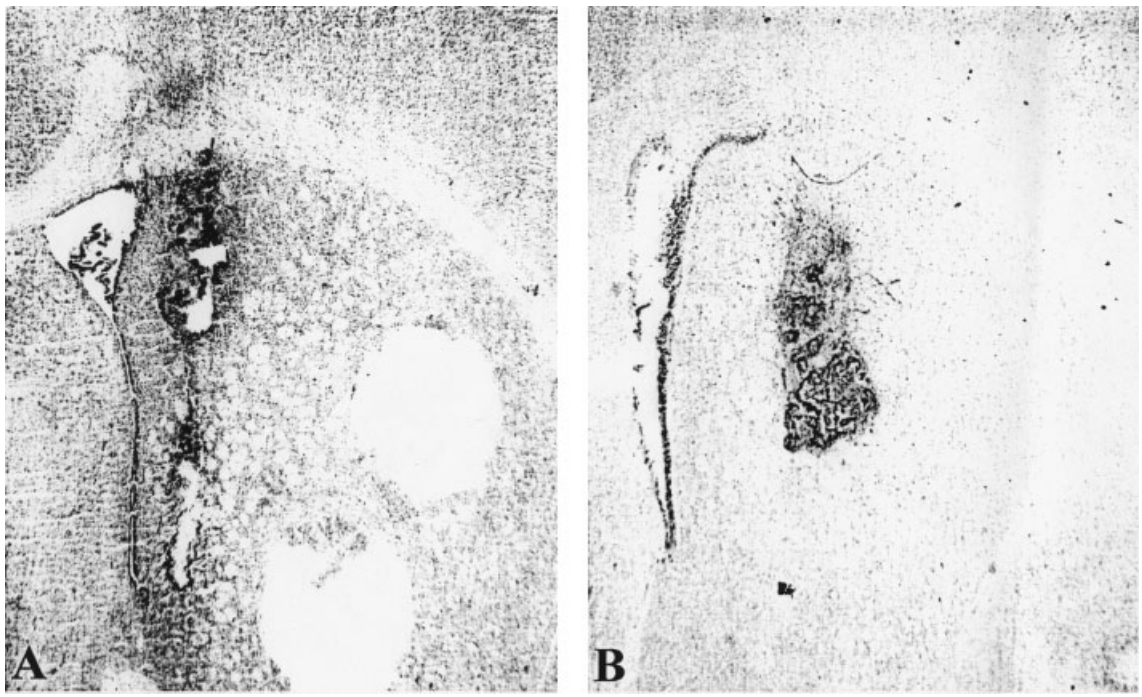

FIGURE 4 Representative photomicrographs of coronal sections from 2 rats treated with 6-OHDA as weanlings. Panel A is from an animal used in Experiment 1 and illustrates the tract of the microdialysis guide cannula and probe in the medial striatum. The guide cannula terminates at the level of the broad aspect of the lateral ventricle, and the gliosis produced by the extension of the membrane tip of the dialysis probe extends more ventrally. The striatal regions that were used for the two micropunches (to quantify tissue DA levels) appear lateral to the dialysis probe. Panel B is from an animal used in Experiment 2 and illustrates the tract of the injection cannula in medial striatum. Microsopical inspection of both sections was conducted using an Olympus AX microscope (Olympus America, Melville, NY). Photomicrographs were obtained using an Olympus Magnafire Digital CCD camera (Model S99806) equipped with a $\frac{2}{3}$-in. chip, attached to the microscope with an Olympus-USPT coupler. Optical magnification was $\times 2$.

representative photomicrograph). Three additional observations are relevant here. First, there were no systematic differences in the striatal placements from rats treated with 6-OHDA or vehicle as weanlings. Second, the general placements of the injection cannula corresponded with the microdialysis probe placements from Experiment 1 (see Figure 4A vs. 4B). Finally, although not depicted in the figure, there were no significant hemispheric differences in the placement of the injection cannula within the striata.

Motoric Behavior Following Intrastriatal Sulpiride.

Akinesia. Baseline measures of akinesia in the two lesion groups were negligible and comparable (Figure 6). Intrastriatal infusion of sulpiride, as expected, produced a dose-dependent increase in akinesia latency, $F(2, 36) = 10.54$, $p < 0.001$, that intensified from 15 to 60 min postinfusion, $F(3, 54) = 17.22$, $p < 0.001$. Most relevant to the issue of lesion-induced supersensitivity, however, is the fact that there was no main effect of lesion nor any significant interaction involving lesion ($ps > 0.05$), suggesting that there were no differences in sensitivity to the impairing effects of sulpiride between DA-depleted and control rats. A restricted number of relevant post hoc comparisons revealed significant differences between basal values and either the low (3.0 μg) or high (10 .0 μg) dose of sulpiride at the 15- and 60-min time points ($ps < 0.05$). The duration of akinesia was greater following administration of 10.0 μg than after 3.0 μg when rats were tested 15 min after infusion ($p < 0.05$).

A similar lack of behavioral supersensitivity to intrastriatal sulpiride was observed with respect to the catalepsy measure (data not shown).

DISCUSSION

These experiments, designed to further characterize differences in the behavioral and neurochemical responses to

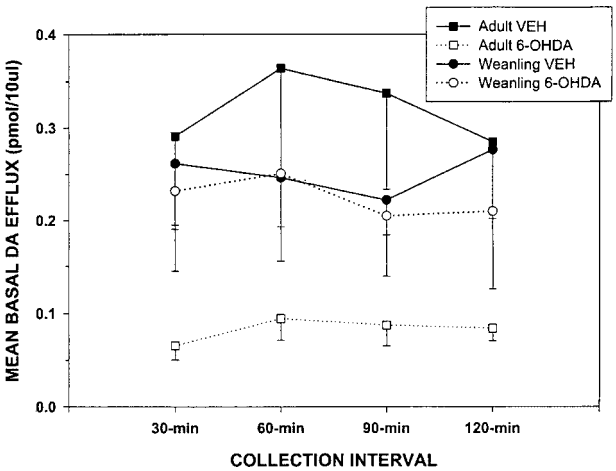

FIGURE 5 Mean (±*SEM*) DA efflux, under baseline conditions, for the four treatment groups. Absolute DA efflux (pmol/10 μl) was corrected for in vitro probe recovery. Microdialysis collections were taken every 30 min, following a 3-hr postprobe insertion equilibration period, and the time point along the abscissa represents the end point of each collection interval. As expected, 6-OHDA treatment in adults resulted in a highly significant reduction in basal DA efflux relative to age-matched vehicle controls that was consistent with the marked DA depletion in tissue (Table 1). Interestingly, basal DA efflux in rats treated with 6-OHDA as weanlings was not significantly different than that seen in age-matched controls despite the fact that tissue levels of DA were as markedly depleted as in adult 6-OHDA-treated rats (Table 1).

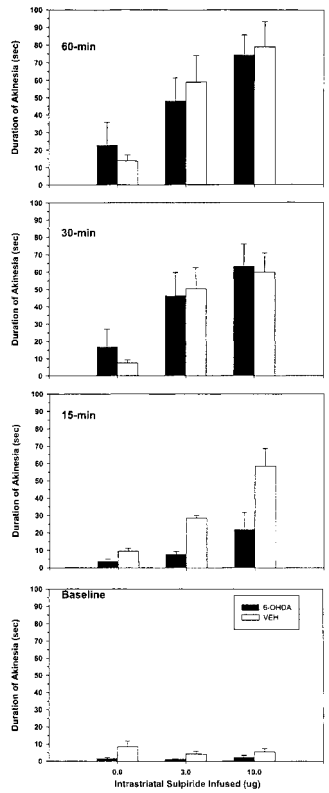

FIGURE 6 Mean (±*SEM*) duration of akinesia (s) in adults treated with vehicle ($n = 12$) or 6-OHDA ($n = 8$) on Day 27. Latencies were determined under baseline conditions and at several time points following the intrastriatal infusion of the D2 antagonist sulpiride. Sulpiride produced dose- and time-dependent increases in akinesia; however, there were no overall differences in the deficits observed in control and DA-depleted rats.

large striatal DA depletions in weanling and adult rats, led to three important observations. First, rats sustaining very large depletions of striatal DA as weanlings were spared from the severe deficits in motor behavior and quickly recovered (i.e., 1–2 days) from reductions in body weight. In contrast, comparably depleted adults exhibited pronounced deficits that were still evident 8 days after the lesion. Second, despite the fact that both age groups had comparable depletions of tissue levels of DA (i.e., ≥95%), there was a marked difference in the levels of extracellular DA as estimated by in vivo microdialysis. In fact, DA efflux in rats depleted of DA as weanlings did not fall outside of the range of control values at a time when efflux in lesioned adults was very much below control values. These age-related differences in DA efflux correlated well with the differences seen in motoric behavior after the lesion. Finally, adult rats depleted of DA as weanlings did not exhibit the behavioral supersensitivity to the debilitating effects of *intrastriatal* administration of DA antagonists that have been repeatedly seen in rats depleted of DA as adults, which may be a reflection of the maintenance of control-like levels of extracellular DA despite large depletions in tissue levels. Each of these observations will now be discussed in more detail.

Age-Dependent Differences in the Acute Effects of Striatal DA Depletions

Rats sustaining large striatal DA depletions as adults were markedly impaired. They stopped eating and required intragastric intubations to maintain caloric and hydrational balance. Despite these efforts, rats still lost weight over the course of the 8 postsurgical days. These animals also exhibited pronounced motoric deficits. They did not move when placed on a flat surface (akinesia) nor when placed in the open field. They also did not extricate themselves when placed in unusual body postures (catalepsy). These behavioral impairments have been repeatedly observed in adult rats with striatal DA depletions (for reviews, see Stricker & Zigmond, 1986, 1989). Although the focus of the present study was on the acute behavioral effects seen during the first week following the lesion, other studies in similarly depleted rats reveal that the partial recovery of independent ingestion and voluntary movement requires several weeks

to months (Stricker & Zigmond, 1986; Zigmond & Stricker, 1989).

In contrast, rats sustaining comparably large striatal DA depletions as weanlings exhibited only a transient decrease in body weight during the initial 1 to 2 days postlesion; however, independent ingestion soon reappeared and rats gained weight by the third day following the lesion—a time when rats lesioned as adults were still aphagic and adipsic. In addition, in striking contrast to the impairments seen in adult rats, the DA-depleted weanlings exhibited normal motoric behavior. It is important to note, when interpreting this remarkable sparing from deficits, that DA transmission is necessary for the expression of sensorimotor behavior in rats during the weaning period as evidenced by the marked deficits induced by the systemic (Weihmuller & Bruno, 1986) or intracranial (Meyer, Cottrell, & Van Hartesveldt, 1993) effects of acute DA antagonists during development.

Age-Dependent Differences in Basal DA Efflux

Basal striatal DA efflux was measured in all four groups on Day 8 postsurgery, a time when ingestive and motoric behavior was still quite different between rats lesioned as adults and the other three groups. DA efflux in rats lesioned as adults was reduced by approximately 80% relative to rats treated with vehicle as adults, and this marked reduction paralleled the obvious persistence in behavioral deficits. The fact that tissue levels suggested a depletion of striatal DA (96–98%) that was greater than that indicated by estimates of extracellular levels of DA is not unexpected. Previous studies have demonstrated that efflux significantly exceeds tissue levels in 6-OHDA-treated adults (Castañeda, Whishaw, & Robinson, 1990), which probably reflects the heightened turnover of DA from residual terminals coupled with the reduction in high-affinity reuptake sites following the lesion (Zigmond et al., 1990).

In contrast, basal DA efflux in rats lesioned as weanlings did not differ significantly from values obtained from age-matched controls, and the absence of a group difference correlated with similar motoric behavior in vehicle- and 6-OHDA-treated rats. The maintenance of normal levels of DA in dialysates from a striatum with tissue levels depleted by 95 to 97% is a novel and remarkable finding, and suggests a degree of compensation within residual DA terminals in weanlings that is apparently not available to rats lesioned as adults. Several other studies have described findings that are consistent with a heightened capacity of developing DA neurons in intact rats or following denervation to function at unanticipatedly high levels. For example, 6-OHDA treatment in neonatal rats (Day 3) that produced a 99% depletion of DA in striatal tissue resulted in only a 12 to 54% reduction, relative to controls, in basal DA efflux (Castañeda, Whishaw, Lermer, & Robinson, 1990). Our present data suggest that the capacity for release and turnover from residual neurons in the denervated striatum from weanlings is even greater than the capacity seen in lesioned neonates. While there may indeed be age-related differences in compensations, it should be noted that rats in the Castañeda, Whishaw, and Robinson (1990) study were tested as adults whereas rats in the present study were tested as weanlings. Thus, the capacity for compensatory release and turnover may diminish as the denervated rats reach adulthood. Another example of exaggerated compensatory mechanisms during development was seen following electrical stimulation of striatal slices taken from intact 10-day-old rat pups. Stimulation resulted in DA release that is greater than that seen in adults when normalized for DA content in tissue (i.e., fractional DA release; Stachowiak, Keller, Stricker, & Zigmond, 1987). Finally, striatal DA turnover, as estimated by DOPAC + HVA/DA ratios, is higher at Day 30 than at Day 56 or 100 (Teicher et al., 1993). Collectively, these data suggest that DA turnover may be higher during certain stages of development than in adulthood. Following a subtotal lesion in weanlings, the residual nigrostriatal neurons may further increase their turnover from this already heightened basal level.

Subsensitivity to the Behavioral Effects of DA Antagonists

Adult rats, treated with 6-OHDA as weanlings, displayed motoric deficits following intrastriatal infusions of the D2 antagonist, sulpiride. This is an important observation, as it reveals that striatal DA receptor activation remains necessary for the expression of normal motor behavior despite the fact that these animals exhibited few, if any, behavioral deficits soon after the lesion. While adults lesioned as weanlings were sensitive to the impairing effects of sulpiride, they were no more sensitive to these effects than were control rats treated with vehicle as weanlings. These results with *intrastriatal* perfusions extend our previous demonstration of comparable sensitivity to the motoric effects of *systemically* administered DA antagonists between rats lesioned as weanlings and controls (Sandstrom & Bruno, 1997), and reveal that striatal DA receptor activation remains necessary for the continued expression of motor behavior in these lesioned animals.

The reactivity of adults depleted of DA as weanlings to direct DA antagonists has been extensively studied and is quite different than that seen in rats comparably depleted as adults. This latter group of animals is supersensitive to the debilitating effects of DA antagonists. Low doses of

DA antagonists, that have no effect in control rats, rapidly reinstate the ingestive and sensorimotor impairments seen acutely after the lesion (Heffner et al., 1977). This marked supersensitivity has been interpreted to suggest that compensations within residual elements of the denervated system in adults may support the protracted and partial recovery of function seen in rats depleted of DA as adults, but these compensations are insufficient to maintain adequate DA receptor activity in the presence of even low concentrations of antagonists. The limitations of this compensation, at the presynaptic level, can be seen in the results of the microdialysis experiment earlier in which values of DA in dialysates were still reduced by 80% in adult lesioned rats.

Two additional points should be made regarding the interpretation of the results of the intrasulpiride experiment. First, rats lesioned as weanlings were tested as adults and not within 8 days of the lesion as in Experiment 1. This choice was influenced by studies conducted on rats depleted of DA as adults. In these studies, rats were tested several weeks to months following the lesion to allow for sufficient recovery of motor behavior so that antagonist-induced deficits can be revealed (Heffner et al., 1977; Stricker & Zigmond, 1986). Therefore, the time interval between the lesion and testing in this article were designed to more closely coincide with previous studies in adult lesioned rats. Second, one must be cautious in attributing normal sensitivity to sulpiride on the basis of only two doses of the drug. However, neither dose produced maximal behavioral effects—thus, there were no limitations due to a potential test ceiling. Moreover, in our previous study, we used three doses of a different DA antagonist and still did not see any signs of behavioral supersensitivity to the drug in rats lesioned as weanlings.

While speculative, the results of the microdialysis experiment may provide a key as to why adults lesioned as weanlings did not exhibit an adultlike supersensitivity to the behavioral effects of sulpiride. Extracellular levels of basal DA in these rats never fell below the range seen in vehicle-treated controls, and thus, there would be no reason to expect a supersensitivity to the effects of sulpiride on motoric behavior. Of course, a more direct test of this relationship would involve a demonstration that rats lesioned as weanlings display a normal sensitivity to sulpiride even when tested only 6 to 8 days postlesion. An important corollary would be the demonstration that an additional striatal DA depletion, within days after the initial depletion, would prevent the sparing phenomenon.

Obviously, an important transition occurs between Day 27 and adulthood in the rat's ability to compensate for large depletions of striatal DA. We have previously determined that this transition occurs by postnatal Day 35 (Weihmuller & Bruno, 1989). 6-OHDA-treatment on Day 35 produces the same prolonged and severe behavioral deficits as those seen in Experiment 1 and in many earlier studies (for a review, see Stricker & Zigmond, 1986). It is relevant to this discussion that rats depleted of DA on Day 35 also exhibit the adultlike behavioral supersensitivity to a DA antagonist (Sandstrom & Bruno, 1997)—suggesting that the limitations in plasticity in the denervated system that resulted in the initial behavioral deficits also were insufficient to maintain DA receptor activity in the presence of even low doses of competitive antagonists.

In conclusion, these experiments demonstrate marked age-related differences in the behavioral and neurochemical responses to large subtotal DA depletions in rats. In contrast to the severe and long-lasting deficits seen in rats depleted of DA as adults, rats comparably depleted as weanlings exhibit few, if any, of the initial behavioral impairments. Moreover, the residual DA neurons in the denervated weanling undergo remarkable compensations that maintain dialysate levels of striatal DA within the control range (despite severe depletions in tissue levels). This compensatory increase in the weanling rats may prevent the occurrence of initial behavioral deficits and eliminate the supersensitivity to the debilitating effects of DA antagonists as the animals mature.

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