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Posture-Independent Sensorimotor Analysis of Inter-Hemispheric Receptor Asymmetries in Neostriatum

TIMOTHY SCHALLERT AND MARGARET UPCHURCH

Department of Psychology

AND

RICHARD E. WILCOX AND DANA M. VAUGHN

Alcohol and Drug Abuse Research Program, Department of Pharmacology and Toxicology
University of Texas at Austin, Austin, TX 78712

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SCHALLERT, T., M. UPCHURCH, R. E. WILCOX AND D. M. VAUGHN. *Posture-independent sensorimotor analysis of inter-hemispheric receptor asymmetries in neostriatum.* PHARMACOL BIOCHEM BEHAV 18(5) 753–759, 1983.— Nigrostriatal dopaminergic neurons are thought to be critically important for somato-sensorimotor behavior. Following unilateral irreversible elimination of these neurons, an animal shows an ipsiversive postural bias and permanently fails to orient its head toward tactile stimuli placed on the contralateral side of the body. In response to apomorphine, a dopamine agonist, these rats display contraversive circling. This effect is thought to reflect denervation-induced proliferation of dopamine receptors in the ipsilateral striatum. We have developed a sensitive procedure that measures sensorimotor function independent of postural and circling biases. We record the latencies to remove small pieces of adhesive stimuli placed onto the snout or radial surface of the forelimbs. The stimuli are placed symmetrically and simultaneously, which is analogous to tactile-extinction procedures used clinically. In the first study we found that rats with unilateral 6-hydroxydopamine (6-OHDA)-induced lesions of the nigrostriatal pathway showed a contralateral sensorimotor bias in response to doses of apomorphine below those necessary to produce contraversive circling. In a second study, unilateral striatal microinjections of kainic acid (KA) were used to destroy the neurons on which the postsynaptic dopaminergic receptors of the nigrostriatal system are contained. Compared to 6-OHDA, KA produced unexpected results in standard orientation tests. None of the KA-treated rats showed contralateral neglect, and some even showed ipsilateral deficits. However, the standard orientation tests are confounded by postural asymmetries, which were irregular in the KA-treated group. Using again the posture-independent sensorimotor procedure, we found that all KA-treated rats, like the 6-OHDA-treated rats, uniformly displayed ipsilateral sensorimotor biases. Sensorimotor function relating to inter-striatal asymmetries may be more specifically assessed with the bilateral-adhesive tests.

Somato-sensorimotor behavior Neostriatum Nigrostriatal dopaminergic neurons

MICROINFUSION of 6-hydroxydopamine (6-OHDA) into the region of the posterior medial forebrain bundle destroys cell bodies, axons, and terminal fields of the mesotelencephalic dopaminergic projections [14]. Animals with severe unilateral damage exhibit postural asymmetry and turning behavior in a direction ipsilateral to the affected hemisphere. In addition to the postural asymmetry, animals with unilateral nigrostriatal damage also exhibit sensorimotor asymmetries. For example, responsiveness to tactile stimulation (von Frey hairs [35]) is abolished, or greatly reduced, contralateral to the affected hemisphere [17,21], and is normal (or enhanced [28]) ipsilaterally.

During the immediate postoperative weeks, dopaminergic receptor density increases notably in the dopamine-depleted neostriatum [5, 9, 32]. This receptor proliferation, which may be a compensatory postsynaptic mechanism that promotes recovery of function by adjusting the net activity in this system, was predicted years earlier from the behavioral work of Ungerstedt [33,34]. He found that dopamine receptor agonists could reverse the direction of postural deviation in these animals and yield exaggerated circling behavior contralateral to the damaged hemisphere. This behavior is similar to that seen when asymmetry in nigrostriatal function is produced via unilateral striatal injections of dopamine [15]. Over the past decade, the "rotational" response rapidly became established as a leading model for studying neostriatal function and drug action. However, it has been difficult to determine whether the direction of sensorimotor bias, like postural asymmetry, can be reversed from ipsilateral to contralateral by apomorphine. Conventional somatosensorimotor assessment relies on the von Frey hair technique, which alone is inadequate for this determination for several reasons. First, the normal sensorimotor response is a head movement in the direction of the tactile stimulus, a behavior that is likely to be influenced by the presence of the well known apomorphine-induced (spontaneous) postural

and turning bias noted above. Second, confusion may arise because the rostral tip of the snout remains highly sensitive to tactile stimuli following unilateral lesions. Thus, should an animal turn "spontaneously" during a test for sensorimotor responsiveness, its nares or most rostral vibrissae might incidently come into contact with the stimulus, and possible subsequent behavior (e.g., biting the stimulus [13,20]) might be misevaluated as a positive response. Finally, the measurement of head orienting to a von Frey hair stimulus is insensitive to sensorimotor asymmetries unless the response on one side in grossly deficient.

These problems can be circumvented by the use of a sensitive sensorimotor test [28] that is an experimental analog of bilateral-stimulation procedures that assess tactile extinction and obscuration in human patients with unilateral parietal or frontal lobe damage [3, 10, 23]. Bilateral stimulation is a method developed first by Loeb in 1884, and introduced into clinical neurology by Oppenheim in 1885 (see Benton [3] for historical review). Patients may readily detect stimuli on either side of the body, provided that the stimuli are presented one at a time. If, however, the stimuli are presented simultaneously, one on each side of the body, the contralateral stimulus is not detected, or feels subjectively less intense. Our bilateral-stimulation analog uses small pieces of adhesive paper as tactile stimuli, which the animals readily remove. This "bilateral adhesive" test compares and quantifies both ipsi- and contralateral tactile responsiveness simultaneously. Because the stimulus-directed adhesive-removing movements do not require lateral head movements, this test works independently of postural asymmetries. For example, we have shown previously that rats with electrolytic, rather than 6-OHDA-induced, lesions of the substantia nigra area exhibit a severe contralateral bias of posture and contraversive circling behavior that coexist with a clear ipsilateral sensorimotor bias [28]. The sensorimotor bias could be detected only by the "bilateral adhesive" test.

It seemed possible that apomorphine-induced contraversive circling might similarly be accompanied by an ipsilateral sensorimotor bias, or that the dose of apomorphine required to reverse postural bias might be different from that which reverses sensorimotor bias. The first study pursued these possibilities.

In a second study, we infused unilaterally into the striatum the heterocyclic glutamate analogue kainic acid (KA), which destroys neuronal somata and dendrites relatively selectively [18, 25, 30]. The postsynaptic dopaminergic receptors of the nigrostriatal system are located on many of these neurons; therefore it seemed reasonable to assume that contralateral sensorimotor integration would be uniformly affected by their elimination. However, intrastriatal kainic acid yielded only irregular effects on stimulus-directed lateral head movements recorded during von Frey hair sensorimotor tests. That is, some animals displayed chronic contralateral biases, some displayed chronic ipsilateral biases, some showed no asymmetry, and some showed mixed effects. Because these orientation tests are contaminated by postural biases, which were irregular in the KA-treated rats, we further examined these animals for regularities in sensorimotor behavior using the posture-independent "bilateral adhesive" test.

METHOD

Subjects

Well-handled male Long-Evans rats (n=38), 300–475 g, were maintained on a 12:12 hr light/dark cycle with free access to food and water.

Surgery

The animals were anesthetized with Equi-Thesin. In the first study, 6-OHDA HCl was infused into the posterior medial forebrain bundle of six rats at a dose of 1.1–1.5 mcl of a 6 mcg/mcl solution (expressed as the salt) and 10 rats were subjected to control operations. Control operations involved infusions of the vehicle, artificial cerebrospinal fluid in a 0.2 mg/ml ascorbate solution (n=6), or simply punctures of the dura (n=4).

In the second study, KA was infused into the striatum of nine rats, 6-OHDA was infused into the nigrostriatal bundle of six rats, and the remaining seven rats received control treatment (see [5,16]). KA was dissolved in 0.9% sodium chloride solution buffered to a pH of 6.5 with a sodium phosphate buffer [18]. The neurotoxin was delivered via a 30 gauge stainless steel cannula connected by polyethylene tubing to a motor-driven (Harvard infusion pump) syringe containing the kainic acid solution. From 0.5 to 1.5 μg KA was administered in a volume of 1.0 μl over a 2-min period. The guide cannula was left in position for an additional two minutes. Supplementary doses of chloral hydrate were given during the first 24 hr after surgery to minimize KA-induced convulsions [2].

Procedure

All behavioral tests were conducted under dim lighting conditions supplemented by red light from a 25 W bulb.

In the first study, a "bilateral adhesive" test was conducted while the animal was alone in its home cage. Small rectangular pieces of adhesive paper (1.5×1.0 cm; Avery International) were applied bilaterally 9-mm caudal to the tip of the snout, and latencies to remove each stimulus were recorded. The degree of stickiness was such that facial hair was not pulled out when the animal removed the adhesive paper.

A battery of other behavioral tests was given during the first few days and again 2–3 weeks following surgery.

Tactile stimulation of the snout, shoulders, forelimbs, and hindquarters was presented by means of a 5-g von Frey hair. Stimulus-dependent head and turning movements in the direction of the locus of stimulation were noted. The summed orientation scores represent each animal's response to von Frey hair stimulation of four separate regions on each side of the body: snout, shoulder, forelimb, and hindquarter. Marshall's five-point scoring system was used [19], where 0=neglect, and 4=precise stimulus-bound head turns with localization and biting of the stimulus.

The direction of negative geotaxis was recorded in two separate tests. In a slanted grid test each animal was placed head down on a 45° grid slope. The direction of turning toward the upper part of the slope was noted [19]. Each animal also was suspended by the base of the tail for 4 sec, and the presence and direction of lateral or rotatory movements of the body were noted [11].

In a test for asymmetrical bracing reactions in the limbs [28], the animals were pushed laterally left and right. At least three trials were given in each direction. Stepping versus bracing reactions to this horizontal displacement were recorded.

All rats were tested for stimulus-independent movements, including postural deviations and circling behavior, in a Plex-

iglas bowl (50 cm diameter). The direction of these relatively "spontaneous" movements was noted [1, 7, 34]. Animals also were placed in a narrow alley (7 cm wide) facing its closed end [8,28]. The direction of rearing or ventral tucking movements which permitted the animal to turn around and face the opposite end was recorded.

Two to three weeks postoperatively, the following doses of apomorphine were delivered cumulatively: 0.000, 0.005, 0.01, 0.05, 0.1 and 0.5 mg/kg SC, each injection being separated by 30 to 40 minutes. Following each injection, the rats were placed in the hemispherical Plexiglas bowls and after ten minutes, circling behavior was analyzed in two 10-minute sessions separated by two 3-min bilateral-adhesive tests in the home cage. During the bilateral-adhesive test also, the presence and direction of asymmetrical posture or circling behavior was noted.

In the second (KA vs. 6-OHDA) study, the bracing test was not conducted, and two additional types of bilateral-adhesive tests were conducted, as described briefly below.

Bilateral stimulation of the radial aspect of the forelimbs (inward, toward the midline as the animal assumes its normal standing posture) was provided by round pieces of adhesive paper measuring 1.3 cm in diameter (Avery International). These stimuli were pressed firmly onto each animal's wrists such that they covered about 1 mm of the hairless part of the forepaw (overlapping most of tactile dermatomes C_6 and C_7). In instances when the adhesive came partially or completely off without the animal having attempted to remove the stimulus with its mouth, the trial was begun again. Care was taken to apply each stimulus with equal pressure, to randomize the order of application (left vs. right) and to gently squeeze both forelimbs simultaneously just before the animal was placed in the home cage for testing. However, it has been our experience that the skin surface area (sensory field) occupied by each stimulus is a far more important consideration than relative stimulus pressure. Sensorimotor bias can be switched readily simply by appropriately adjusting the relative size of the ipsilateral vs. contralateral adhesive stimuli. In contrast, even if application pressure of the stimulus on one side of the body or the other purposely is made asymmetrical, this typically does not alter sensorimotor bias, as long as the sizes of the two stimuli are equal.

Bilateral stimulation of the ulnar (outward) aspect of the forelimbs also was provided by the 1.3 cm circular adhesive stimuli, which covered the more caudal tactile dermatome T_1 and part of C_8.

TABLE 1

BILATERAL-ADHESIVE TEST: AVERAGE LATENCIES TO REMOVE ADHESIVE STIMULI FROM IPSILATERAL VERSUS CONTRALATERAL SNOUT, WITH AND WITHOUT APOMORPHINE

	ipsi	contra
	6-OHDA Group	
0.00 mg/kg	12.33 ± 6.0	30.50 ± 14.6
0.01 mg/kg	34.28 ± 14.9	16.28 ± 6.8
	Control Operations	
0.00 mg/kg	22.35 ± 13.9	15.15 ± 7.6
0.01 mg/kg	26.92 ± 11.5	22.71 ± 11.0

Data are means ± S.E. In the 6-OHDA-treated group, all six rats showed ipsilateral bias without apomorphine ($p<0.03$, sign test). This bias was reversed to a contralateral bias by the administration of apomorphine at a dose of 0.01 mg/kg (in 83.3% of these rats) or 0.05 mg/kg (in 100% of these rats; $p<0.03$, sign test). Fisher exact probability test indicated that the 6-OHDA-treated group was significantly more likely to reverse its undrugged sensorimotor bias in response to apomorphine than was the control group ($p<0.05$).

Neurochemical Analysis

High performance liquid chromatography. Determination of dopamine depletions was carried out as previously described [12,36]. The 6-OHDA-treated rats in the second study were sacrificed and striatal tissue was sonicated and stored at −80°C in 200 μl 0.2 N H_3PO_4 containing 1 mM $Na_2S_2O_4$ and 10 ng 3,4-dihydroxybenzylamine (DHBA). Catecholamines were adsorbed onto 10 mg Al_2O_3 in 0.5 M Tris buffer (pH 8.6) by shaking on ice for 10 min. Following three rinses with distilled water (containing 200 μl 0.5 M Tris buffer per 200 mL H_2O) catechols were eluted into 200 μl of cold 0.2 N H_3PO_4 (containing $Na_2S_2O_4$) by shaking on ice for 10 min. Typical recoveries of extracted standards were >70%. Twenty-μl portions of the eluant were injected into a high performance liquid chromatography (HPLC) system (reverse phase with electrochemical detection). The chromatographic system consisted of a Beckman model 112A pump, model 210 injector valve (50-μl sample loop) and a reverse phase 5-micron ultra sphere C18 column (0.45×25 cm). The column eluant was monitored via a Bioanalytical systems amperometric detector Model LC4A with the detector potential set at +0.65 V using a glassy carbon working electrode. A 4-cm precolumn packed with C18 packing protected the analytical column. Mobile phase flow rate was 1.3 ml/min. The LC4A detector (sensitivity set at 2 nA) was connected to a Shimadzu CR1A data processor for initial peak processing.

The mobile phase (pH=4.0) consisted of 0.83 M NaH_2PO_4, 0.083 mM Na_2EDTA, 0.1 mM sodium octylsulfate and 8.3% HPLC grade methanol. With a 1.3 ml/min flow rate retention times were l-dopa 3.3 min, norepinephrine 4.4 min, DHBA (internal standard) 6.3 min and dopamine 9.9 min.

(^3H)-Spiroperidol binding. To estimate the effectiveness of the KA microinfusions, spiroperidol binding studies were carried out as previously described [27,37]. Striatal homogenates previously stored in 50 mM Tris buffer (pH 7.7,containing 0.1% ascorbate) were thawed and pipetted into cold centrifuge tubes. The vial in which the tissue was stored was washed with 3 ml Tris buffer (pH 7.7) containing 0.1% ascorbate. This wash was added to the tissue homogenates which were then centrifuged (50,000 ×g) for 10 min. The pellets were washed in 4 ml pH 7.7 Tris buffer, recentrifuged and then suspended in 4 ml 50 mM Tris buffer (pH 7.1) containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. These homogenates were centrifuged for 10 min at 50,000 ×g, the supernatants removed, and the pellets resuspended in 4 ml of the pH 7.1 Tris buffer.

This tissue preparation yielded a protein concentration of approximately 400 μg per incubation tube. For determination of total ligand binding triplicate tubes contained tissue, 50 mM Tris (pH 7.1 plus ions) and (^3H)-Spiroperidol (0.2 nM/tube; Kd=0.23 nM; 26 Ci/mmol, New England Nuclear). For determination of nonspecific ligand binding, triplicate

TABLE 2

UNILATERAL STRIATAL KAINIC ACID: EFFECTS ON SENSORIMOTOR AND MOTOR ASYMMETRIES AND ON STRIATAL (^{3}H)-SPIROPERIDOL BINDING

	% Decrease	Bilateral-Adhesive		Von Frey		Motor Tests		
Rat	Spiro binding	Ipsi	Contra	Ipsi	Contra	Grid	Alley	Tail
14	60.8	15.4	88.0	14	15	C	C	C
17	76.0	9.2	11.7	16	16	N	C	C
18	42.0	17.6	30.1	16	16	I	N	N
19	86.8	17.0	27.0	13	10	N	N	C
12	57.5	10.0	30.0	15	15	I	N	N
48	73.8	5.0	79.0	4	16	I	I	C
49	80.8	13.9	23.5	14	12	C	C	C
40	55.1	6.7	72.2	0	15	C	C	C
41	54.7	5.3	15.4	0	16	C	C	C
Mdn	60.8%	10.0*	30.0	14	15			

(a) Percent decrease in (^{3}H)-spiroperidol binding in damaged striatum relative to contralateral striatum, (b) Latency (in sec) to remove adhesive stimuli applied bilaterally to radial aspect of forepaw, (c) Summed orientation score in von Frey hair sensorimotor test (16 = maximal normal response), (d) Biases in the motor tests (I = exclusively ipsilateral; C = exclusively contralateral; N = no consistent bias).

*$p<0.01$, significantly different from contralateral (sign test).

tubes contained the same as above plus 1 μM d-butaclamol. After a 15-min incubation at 37°C, the contents of each tube were filtered under vacuum through Whatman GF/B glass microfilters. The filters were rinsed three times with 5 ml ice-cold Tris buffer (pH 7.7) and counted 24 hr later by liquid scintillation spectrometry (Beckman LS 9000; efficiency=40%). Specific ligand binding was determined by subtracting nonspecific binding from the total bound radioactivity.

RESULTS

After at least 2 postoperative weeks, every 6-OHDA-treated rat consistently displayed postural and movement asymmetries suggesting severe unilateral depletion of striatal dopamine [1, 17, 19, 21, 28, 29]. That is, in the absence of apomorphine, they spontaneously turned only in the direction ipsiversive to the affected hemisphere, and failed to make head turns toward a 5 g von Frey hair stimulus placed anywhere on the contralateral side of the body. The animals consistently turned around ipsiversively at the closed end of an alleyway. They turned 180° ipsilaterally when placed head down on an inclined grid, and they flexed or rotated their trunk immediately ipsilaterally when they were suspended by the base of the tail. When pushed in a straight line sideways in the direction contralateral to the damage, the animals actively resisted displacement by means of bracing reactions in the limbs, but made rapid stepping or hopping reactions when pushed in the ipsilateral direction.

Without apomorphine, there was consistent ipsilateral adhesive removal bias in every 6-OHDA-treated rat (Table 1). As a group, and individually, the latencies to remove the ipsilateral stimulus were shorter than the latencies to remove the contralateral stimulus ($p<0.03$). It is important to note that although these rats chronically failed to make lateral head turns directed toward von Frey hair stimulation of the contralateral whiskers (neglect), after 2 weeks they readily removed contralaterally placed adhesive stimuli following removal of ipsilaterally placed stimuli [28]. Thus, stimulus-directed behavior that did not involve lateral head movements remained chronically intact on both sides, but the sequence was asymmetrically biased. The ipsilateral adhesive-removing bias could be shifted to contralateral in each rat simply by increasing the size of the contralateral stimulus and/or decreasing the size of the ipsilateral stimulus (Mdn contra/ipsi ratio=5:1). This effect emphasized the sensory, over the postural, nature of the asymmetries.

Apomorphine produced a contralateral sensorimotor bias in every 6-OHDA-treated rat (but not in rats with control operations) as measured by the bilateral-adhesive task ($p<0.03$, Table 1). These data confirm and extend the work

TABLE 3

LATENCY (IN SEC) TO REMOVE BILATERAL ADHESIVE STIMULI APPLIED TO THE SNOUT IN KAINIC ACID TREATED RATS

Rat	Ipsi	Contra
14	1.4	2.3
17	34.1	13.7
18	21.0	23.0
19	2.4	8.4
12	1.5	18.4
48	4.8	8.6
49	2.1	5.2
40	5.0	7.0
41	24.0	24.0
Mdn	4.8*	8.6

*$p<0.03$ (one tailed).

TABLE 4

LATENCY TO REMOVE ADHESIVE STIMULI PLACED ON THE ULNAR ASPECTS OF THE FORELIMBS IN 6-OHDA, KAINIC ACID, AND SHAM TREATED RATS

	Ipsi	Contra
6-OHDA	13.8*[a]	26.7
	(3.9–31.6)	(14.0–300)
Kainic	12.2*	38.0
	(3.7–35.4)	(9.1–240.0)
Control	37.3	34.3
	(9.6–121.3)	(8.3–90.8)

[a]Scores represent median latency (sec) with ranges in parentheses.
*$p<0.05$ (two tailed), significantly different from contralateral.
Every rat in the KA and 6-OHDA groups removed the ipsilateral stimulus first on all trials.

TABLE 5

LATENCY FOR KAINIC ACID TREATED RATS TO FIRST CONTACT IPSI- OR CONTRALATERAL FORELIMB DURING RADIAL VERSUS ULNAR BILATERAL-ADHESIVE TESTS

	Ipsi	Contra
Radial	5.0*[a]	15.0
	(3–15.5)	(3–41.5)
Ulnar	3.0*	10.9
	(2–30)	(6–40)

[a]Data are mdn latencies (sec) with range in parentheses.
*$p<0.05$, significantly different from contralateral.

TABLE 6

LATENCY FOR 6-HYDROXYDOPAMINE TREATED RATS TO FIRST CONTACT IPSILATERAL OR CONTRALATERAL FORELIMB DURING RADIAL VERSUS ULNAR BILATERAL-ADHESIVE TESTS

	Ipsi	Contra
Radial	6.3*[a]	11.8
	(1.5–14.0)	(3.0–75.0)
Ulnar	6.9*	37.1
	(4.0–18.9)	(3.0–34.0)

[a]Data are mdn latencies (sec) with range in parentheses.
*$p<0.05$, significantly different from contralateral.

of others [13,20]. It is important to note that this reversal of asymmetry occurred in five of the six rats at 0.01 mg/kg, a dose that was too low to produce any contralateral circling behavior (all circling was ipsilateral at this dose in all rats). The remaining rat showed reversal of sensorimotor asymmetry at 0.05 mg/kg. At a lower dose (0.005 mg/kg), three of the six animals showed a consistent contralateral sensorimotor bias on apomorphine trials. These low doses are reminiscent of doses of apomorphine that are suspected of being autoreceptor-specific doses [26]. However, autoreceptor-induced negative feedback in the intact hemisphere ought to be balanced by the absence of dopaminergic terminals in the damaged hemisphere.

At the higher doses (0.05, 0.10 and 0.5 mg/kg), these animals progressively showed stereotyped licking, gnawing and circling behavior, but often failed to contact or remove the adhesive stimuli. We believe that the higher doses may have interfered with the neural integration necessary for this type of coordinated stimulus-guided movement. High doses of amphetamine (greater than 4 mg/kg) also interfere with adhesive removal (Schallert, unpublished data). Although one might argue that the postural and circling behaviors simply were incompatible with the sensorimotor behavior, in previous work we have observed consistently that undrugged animals readily remove the adhesive stimuli despite severe circling in a direction opposite to the adhesive-removal bias (in rats with nigra/electrolytic lesions [28]), ataxia (in rats with severe unilateral inner ear disease; Schallert, Spirduso and Upchurch, unpublished data), or intense stereotyped scanning behavior seen in rats treated with 50 mg/kg atropine sulfate (Schallert and Farrar, unpublished data).

At 0.05 mg/kg, only three of the 6-OHDA-treated rats showed spontaneous (stimulus-independent) contralateral circling behavior. At the highest doses (0.1 and 0.5 mg/kg), five of these six rats showed rapid and exclusive contralateral circling behavior (greater than 7 turns/min), whereas none of the control animals displayed circling.

As noted above, the typical dose of apomorphine at which the rats showed a reversal of sensorimotor bias, compared with circling bias, was quite different. Moreover, one animal that showed a reversal of sensorimotor bias failed to show a reversal of circling bias at any dose of apomorphine. It may be important that the reversal of sensorimotor asymmetry occurs independently of the reversal of postural and circling asymmetry. However, for now we are unable to conclude with certainty that the underlying anatomy or receptor physiology in the striatum is fundamentally different for apomorphine-induced "sensorimotor" versus "postural" functions. Instead we simply stress that these two types of behaviors can be measured independently and that with further experiments, their neural organization or mechanism for adaptation to damage to their neural substrates might ultimately turn out to be different.

In the second study, five of the 6-OHDA-treated rats had 90% or greater ipsilateral depletions of striatal dopamine relative to contralateral levels, as determined by HPLC (Mdn=93.9%; range=75–99%). As expected, these animals had strong ipsilateral biases as demonstrated by head orientation and postural tests (Mdn ipsilateral von Frey hair-orientation score=16, the maximum possible; contralateral=0).

Each 6-OHDA-treated rat removed the ipsilateral radial stimulus before it removed the contralateral stimulus on all trials ($p<0.03$; latencies for the first trial of the radial adhesive tests: Mdn=10.4 sec, ipsilateral vs. 18.8 sec contralateral). In contrast, only one of the seven control rats removed consistently the ipsilateral stimulus first and only four rats removed the ipsilateral stimulus first on the first trial (latencies: Mdn=38.6 sec ipsilateral vs. 40.9 sec, contralateral). The differences in adhesive-removal performance between 6-OHDA and control animals were significant ($p<0.05$, Chi-square test). Table 4 confirms these results with ulnar placements of adhesive.

Unilateral microinjections of KA into the striatum did not result in a general ipsilateral bias in the slanted grid, alley and tail-suspension tests (Table 2). The behavioral responses

of the rats several weeks after the lesion was made indicated either no consistent bias, or, in four animals, a contralateral bias. Two of the contralaterally-biased rats exhibited total neglect of the von Frey hair stimulus on the ipsilateral side.

Despite their performance on the von Frey hair and motor tests, the rats exhibited ipsilateral bias in response to bilaterally-applied radial (latencies: Mdn ipsilateral=10.0 sec vs. contralateral=30.1; Table 2), ulnar (Mdn ipsilateral=12.2 sec vs. contralateral=28.0; Table 4), and snout (Mdn ipsilateral=4.8 sec vs. contralateral=8.6; Table 3) stimuli. The results obtained when stimuli were placed on the snout were marginal. The tied score resulted from simultaneous removal of both stimuli, which does not occur with the radial or ulnar placements.

The ipsilateral bias apparent in rats with 6-OHDA or KA-induced lesions was probably not due to the greater efficiency of adhesive removing movements (e.g., pulling upward on the adhesive) on the ipsilateral side relative to the contralateral side. Tables 5 and 6 show latency to contact ipsilateral versus contralateral forelimbs with the mouth in KA-treated and 6-OHDA-treated rats. These data ignore the efficiency of that movement and (in the case of ulnar placements) rotation of the limb. The data indicate that treatment with either type of neurotoxin produced an ipsilateral bias in latency to contact the forelimbs.

Severe decreases in (^{3}H)-spiroperidol binding (Table 2) confirmed that relative to the contralateral striatum, all of the rats treated with KA lost striatal tissue with dopamine receptors ipsilaterally. There was no indication of receptor proliferation on the lesioned side. Three randomly selected rats given apomorphine showed no change in sensorimotor bias. Their turning bias with apomorphine treatment was, as expected, exclusively ipsiversive.

The postural effects of KA seen in some of the undrugged rats were reminiscent of the contraversive behavior following KA or electrolytic lesions of the nigra [6, 24, 28, 31]. The apparent differences between 6-OHDA and KA may be due to a differential (relative) specificity of the two neurotoxins. Most importantly, KA-induced postural effects may have interfered with some animals' ability to make stimulus-directed head movements toward von Frey hair stimulation on the ipsilateral side. Perhaps non-dopaminergic strionigral neurons are involved in the expression of the contralateral postural/motor bias [15,22], while nigrostriatal dopaminergic pre- and postsynaptic neurons are involved in the adhesive removal task. We do not know the extent to which the behavioral effects observed after the KA infusions were influenced by regional variations in striatal cell destruction or the postoperative time of behavioral analyses (see [31]). We also do not know the extent to which the effects were caused by nonspecific changes in nigrostriatal dopamine terminals, in striatal cells that do not contain dopamine receptors, or in neuronal or supporting elements outside the region of the striatum [30,38]. However, it seems reasonable to contend that KA and 6-OHDA yielded similar effects in the bilateral-adhesive sensorimotor tests because they each disrupted synaptic function in the nigrostriatal system.

These data have relevance to the studies of Bjorklund and his colleagues [4], who use tests of motor behavior to evaluate the success of their nigrostriatal transplants. In their work, embryonic nigral tissue is implanted into the dopamine-depleted striatum of 6-OHDA-treated adults. Transplant-related insult to, or miswiring of, intrinsic neurons of the striatum could potentially restore postural symmetry in 6-OHDA-lesioned rats, yet worsen or fail to decrease sensorimotor asymmetry, as measured by the bilateral-adhesive test. Because the effects of the nigral transplant on posture in rats without 6-OHDA lesions has yet to be systematically investigated, there is no reason to rule out the possibility that nigral transplants might actually hinder, rather than restore striatal function.

DISCUSSION

Nigrostriatal dopaminergic neurons are thought to be critically important for somato-sensorimotor behavior. Following unilateral irreversible damage to these neurons by 6-OHDA, an animal permanently fails to orient its head toward tactile stimuli placed on the contralateral side of the body [17, 19, 21, 28]. In the present experiments, two different neurotoxin treatments were compared, both of which severely interfered with nigrostriatal function unilaterally. Some rats were given microinfusions of 6-OHDA into the medial forebrain bundle, and others were given KA into the striatum. KA produced unexpected results. Although all of the 6-OHDA-treated rats displayed contralateral neglect of von Frey hair stimuli, none of the KA-treated rats showed contralateral neglect, and some even showed ipsilateral orientation deficits. However, standard orientation tests are confounded by postural asymmetries, which were irregular in the KA-treated group (the asymmetries were ipsiversive in the 6-OHDA-treated rats). Therefore, a more sensitive procedure was used that measures sensorimotor function independent of postural bias. The latencies to remove small pieces of adhesive stimuli placed bilaterally onto the forelimbs or snout were recorded. Rats in both the KA and 6-OHDA groups uniformly removed the ipsilateral stimulus first, and then removed the contralateral stimulus. Thus, the problem of the unexpected results of KA was resolved. Both groups showed an ipsilateral sensorimotor bias in the bilateral-adhesive test.

In the 6-OHDA-treated rats, moderate challenge doses of the dopamine agonist apomorphine shifted their ipsilateral bias to contralateral, and at doses below those necessary to produce the well known apomorphine-induced circling response.

These results demonstrate that sensorimotor function following manipulation of striatal activity is more specifically assessed with a battery of tests that includes the posture-independent adhesive-removal procedure, and that the procedure may be valuable as a quantitative assay for interhemispheric asymmetries in sensorimotor-related dopaminergic receptor sensitivity.

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