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Tactile Extinction: Distinguishing Between Sensorimotor and Motor Asymmetries in Rats with Unilateral Nigrostriatal Damage[1,2]

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SCHALLERT, T , M UPCHURCH, N LOBAUGH, S B FARRAR, W W SPIRDUSO, P GILLIAM, D VAUGHN AND R E WILCOX *Tactile extinction Distinguishing between sensorimotor and motor asymmetries in rats with unilateral nigrostriatal damage* PHARMAC BIOCHEM BEHAV **16**(3) 455–462, 1982 —We describe and demonstrate the usefulness of a reliable procedure for quantifying "sensorimotor" asymmetries in rats with nigrostriatal damage Conventional tests, such as the popular Von Frey hair technique are sometimes inadequate in that positive responses are difficult to quantify, they are susceptible to subjective interpretation, and they require that the animal make head movements Circling behavior has become established as a measure of asymmetrical nigrostriatal activity, but it does not measure stimulus-directed movement One group of rats was given unilateral microinfusions of 6-OHDA into the nigrostriatal system which decreased levels of dopamine in the ipsilateral striatum and yielded an ipsilateral turning bias Another group was given electrolytic lesions in the substantia nigra area, which yielded a severe contralateral circling bias Small pieces of adhesive paper were applied to various parts of the limbs or snout and latencies to remove the stimuli were recorded Because head and body movements were not required, assessment of stimulus-directed movement asymmetries could be quantified rapidly without the interference of stimulus-independent ('spontaneous") postural and motor asymmetries In the 6-OHDA-treated rats there was ipsilateral bias in stimulus-directed activity Indeed, there was ipsilateral hyper-reactivity relative to sham controls In rats with electrolytic nigra lesions, despite a strong contralateral "spontaneous" motor bias, there likewise was an ipsilateral bias in stimulus-directed movement, which could only be detected using the adhesive removal test This similarity in stimulus-directed movement asymmetry between the two groups may reflect a common asymmetry in ascending dopaminergic pathways The opposite circling bias following electrolytic nigra lesions may reflect differential damage to non-dopaminergic efferent projections The bilateral adhesive removal (tactile extinction) test appears to permit the separate quantification of stimulus-directed and stimulus-independent movement asymmetries

Sensory extinction 6-Hydroxydopamine Sensorimotor dysfunction Catecholamines Asymmetry Neostriatum Substantia nigra Neglect

FOR many years investigators have speculated about the neural sources of asymmetrical movements in animals In his classic textbook in 1915 [39], Luciani covered more than a century of prior research on the asymmetrical behavioral effects of unilateral destruction of various brain areas In 1918, Loeb [38] reviewed behavioral tropisms and taxes, and later, Fraenkel and Gunn [16] wrote on the concept of "reflex maps" for unlearned orientation behavior More recently, Denny-Brown [11] discussed the hierarchical organization of forced movements in man

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[2] Sketches that illustrate the placement of stimuli will be attached to each reprint requested

In recent investigations of brain function, much attention has been paid to intrinsic and experimentally imposed interhemispheric neuronal asymmetries [10, 20, 56, 68, 76] In rodents, the nigrostriatal dopaminergic system has been the substrate most extensively studied, and there is good evidence that asymmetries in "spontaneous" and dopaminergic drug-induced movement preferences exist in neurologically intact animals [18, 20, 32, 55] Focus on the nigrostriatal system grew primarily out of the work of others over the past decade (e g , [1, 2, 40, 44, 45, 71, 72, 74]) who have experimentally altered the activity of ascending dopaminergic pathways and postsynaptic function For example, unilateral chemical or electrical stimulation of the nigrostriatal system yields contralateral circling behavior and reduced thresholds for stimulus-directed behaviors on the contralateral side Also, unilateral denervation of the origins and terminal fields of ascending catecholaminergic pathways yields an ipsilateral turning bias and a contralateral deficit or loss of stimulus-directed head movements (also called sensory inattention, neglect or sensorimotor impairment) According to some (e g , [37]), the somato-sensorimotor deficits are permanent if the denervation is essentially complete, which indicates that the nigrostriatal system in one hemisphere may function as a critical link between contralateral tactile input and normal orienting responses

There are several hurdles blocking rapid progress in this area of research Although clearly an advancement 10 years ago, conventional tests which assess stimulus-directed movements, such as the popular Von Frey hair technique [27, 37, 45, 60], are sometimes inadequate in that they are difficult to quantify and are highly susceptible to subjective interpretation Moreover, as in all presently available measures of stimulus-directed behavior involving head movements, the chance that the assessment is due partly, or even chiefly, to "motor" rather than "sensory" or "sensorimotor" asymmetries is a source of concern Elaborate instrumental conditioning and discrimination tests have been devised by a few investigators [7, 14, 27, 70] in attempts to deal with the problem, but these are painstaking, somewhat controversial [34], and, because of the learning component, are undesirably complex and sometimes difficult to interpret

Circling (rotational) behavior has become established as a useful and readily quantifiable measure of asymmetrical nigrostriatal activity, but it also suffers from major limitations First, quantification of circling behavior does not indicate whether the movement is stimulus-independent (i e , the source of the movement is unknown) or stimulus-directed Second, circling may be caused, or modified by alterations in striatonigral or other nearby non-catecholaminergic efferent systems [4, 6, 13, 26, 30, 31, 36, 41, 46, 47, 48, 50, 51, 52, 68, 69, 75] Indeed, nonspecific electrolytic lesions in the substantia nigra pars reticulata area, or slightly caudal in the reticular formation, cause animals to circle tightly "spontaneously" in a direction *contralateral* to the damaged side [8, 29, 31, 39, 61, 64], which is opposite to the ipsilateral turning and sensorimotor bias of the drug-free 6-OHDA-treated animal Such circling, which appears to be stimulus-independent, could be expected to interfere with the assessment of the directional bias and relative strength of stimulus-directed (sensorimotor) movements

Loeb [38] suggested long ago that measurement of the strength and direction of stimulus-guided movement should be done relative to an antagonistic stimulus In the present experiment we describe a new and extraordinarily simple tactile extinction technique for reliably quantifying stimulus-directed movement asymmetries which potentially can be related to the nigrostriatal system in rats Small pieces of adhesive paper were applied to the surface of the body bilaterally at given loci (e g , the snout or dermatomes C_6–C_7 of the forepaws), in unilateral 6-OHDA-treated rats and in rats with electrolytic substantia nigra lesions Latency to remove each stimulus was recorded Because head movements were not required, assessment of alternative stimulus-directed movement asymmetries could be quantified rapidly without the interference of stimulus-independent postural and motor asymmetries

METHOD

Animals

Forty-six male Long-Evans hooded rats were maintained on a 12 12 hr light/dark cycle with food and water available ad lib Twelve rats were group-housed, four to a cage, in clear plastic cages with wire mesh floors Fifteen rats were maintained in individual wire mesh cages An additional 19 rats were group housed prior to surgery and moved to individual cages afterwards All animals were handled daily

Surgery

MFB/6-OHDA The rats were anesthetized with equithesin (0 25–0 35 cc/100 g) 6-Hydroxydopamine (6-OHDA) was infused into the left (n=16) or right (n=14) medial forebrain bundle (MFB) just anterior to the substantia nigra in a vehicle of artificial cerebrospinal fluid with 1% ascorbic acid in a concentration of 6 μg/μl The infusion volume ranged from 0 75 to 2 μl, and the infusion rate was 1 μl/min The coordinates with skull horizontal between lambda and bregma, were 3 2 mm posterior and 1 5 mm lateral to bregma, and 7 2 mm below the dura of the cortex Control (sham) operations were performed on 10 rats Six of these were infused with vehicle only, and four of these sustained punctures of the dura without subsequent infusion

Substantia nigra/electrolytic lesions Six additional rats received electrolytic lesions in the substantia nigra area (4 9 mm posterior and 2 2 mm lateral to bregma, and 7 2 mm ventral to the dura, 1 mA anodal current for 20–30 sec) Three received lesions on the right side of the brain and three received lesions on the left

Procedure

Adhesive paper tests In pilot work we found that a small piece of mildly sticky adhesive paper, placed on the body (e g , various parts of the snout, forepaws or hindpaws) will be rapidly removed by the rat Latency to initiate a stimulus-directed movement, and latency to remove the stimulus from the body can be quantified readily In the present study, the adhesive stimuli were placed on various loci bilaterally so that asymmetries in responsivity, both major and minor, could be measured Thus, the tests resembled the so-called "extinction" task in human patients with neurological disorders [62] The tests were performed in the home cage two to six days after surgery For group-caged animals the cage mates were removed during testing

For tactile stimulation of the snout, strips of adhesive paper that are commonly used as labels for file folders (Avery), were cut into 1 9 cm × 1 4 cm rectangular pieces These pieces were placed lengthwise (horizontal to the ground) along each side of the snout, with the forward edge about 6 mm behind the nose (it was helpful to color-code left from right) The vibrissae were lightly pressed against the snout (In our pilot studies we observed that the labels in a few packages were too sticky for the rats to remove without pulling out some of the fine facial hair Although this did not prevent removal of the stimulus, the degree of stickiness need not be so great, and it can be adjusted by repeatedly pressing the paper onto the palm of the experimenter's hand and then peeling it off) Latency to remove each stimulus was recorded along with a description of the stimulus-directed movements Each trial ended when both labels were removed, or after three minutes Two to five trials were given, but only the first trial was used in the data analyses

Bilateral tactile stimulation of the forelimbs, in 2 separate tests at different loci, was provided by round pieces of adhesive paper ("dots") measuring 1 3 cm in diameter (these "dots" are manufactured by Avery International and are used to color-code file folders) The "dots" were placed bilaterally on the radial aspect (inward, toward the midline as the animal assumes its normal standing posture) of the animal's wrists The distal part of the dot covered about 1 mm of the hairless part of the forepaw This placement overlapped approximately the rostral rootlets area of the tactile dermatomes C_6 and C_7, but left the C_5, C_8 and T_1 areas virtually, or completely, uncovered [25]

To minimize possible variations in adhesiveness caused by lipids in the fur and skin, the forelegs of the rats were washed with a 50% ethanol solution and allowed to dry for at least 15 min before testing Before the "dots" were put on, the wrists and paws were wiped with a clean, dry cotton gauze

Although to remove the stimulus, normal animals often move their heads slightly laterally and downward toward the forepaw, a head movement is not required, and indeed, is often not performed The forepaw can be brought upward and forward to the mouth, which permits the animal to pull the dot off efficiently without a head movement

In a separate test, the dots were placed on the ulnar (outward) aspect of the animal's wrist, which overlapped approximately with the more caudal tactile dermatome T_1, but also included part of C_8 The normal movement used to remove the more caudal stimulus involved a 60 to 90 degree rotation of the forelimbs Latency to contact each foreleg with the mouth, and latency to remove each dot from the wrist were recorded Other behaviors, such as bilateral "face-washing" or rapid paw shakes also were noted, however, these movements were not considered to be stimulus-directed paw contacts The trial ended when the animal removed both dots, or after 300 sec elapsed Occasionally, a dot fell off without the animal having made a stimulus-directed response In such cases, the trial was begun again Because the nigra-lesioned rats were generally less responsive to the dots on the forepaws, the larger, 1 9 cm × 1 4 cm rectangular pieces of adhesive papers were used for this group in a way that tactile dermatomes C_6 through T_1 were stimulated

For tactile stimulation of the hindpaws, the rectangular adhesive paper was pressed over the top of the foot and folded over its inward (tibial) aspect (L_4 to L_5 dermatomes) The stimuli were placed bilaterally As described above, the hindfeet were washed with alcohol and wiped with clean, dry cotton gauze to remove lipids before the stimuli were applied Latency to contact each hindpaw and the latency to remove the rectangular stimulus from each paw were recorded The trial ended when the animal removed both stimuli, or after 600 sec elapsed

Von Frey hair tests Light tactile stimulation of the snout or shoulders was presented by means of 5 g Von Frey hairs [45, 60, 70, 78] Stimulus-dependent head and turning movements directed toward the locus of stimulation or withdrawal movements were noted

Negative geotaxis The direction of negative geotaxis was recorded in two separate tests The first test involved placing the animal on a 45° grid slope in 3 different positions head down, ipsilateral side down, or contralateral side down The direction of turning toward the upper part of the slope [42] was noted The second test involved briefly suspending the animal by the base of the tail The presence and direction of lateral or rotatory movements of the body (trunk flexion) were noted [27]

Bracing Asymmetrical bracing reactions in the limbs were recorded [12,59] When pushed in a straight line sideways in the direction contralateral to the damage, 6-OHDA-treated rats actively resist horizontal displacement by means of a bracing reaction When pushed ipsilaterally, the animals readily make rapid stepping or exaggerated hopping movements, most frequently with the ipsilateral forelimb

Stimulus-independent ("spontaneous") behavior All rats were tested briefly for asymmetrical movements that were apparently non-directed, or relatively "spontaneous" These tests were selected because previous work has shown that ipsilateral movement is a reliable indicator of severe unilateral striatal depletion in 6-OHDA-treated animals The direction of spontaneous circling ("rotation") was recorded (e g , [1, 20, 61])

In addition, the animals were placed in a narrow alley (7 cm wide) facing its closed end [21,58] The direction of rearing or ventral tucking movements which permitted the animal to turn around and face the opposite end of the alley, was recorded

Histological Analysis

The nigra-lesioned (electrolytic) animals were given an overdose of equithesin and were perfused with saline followed by a 10% formal-saline solution Their brains were extracted and frozen sections (40 μm) throughout the lesion were mounted and stained with thionin Histological analysis was aided by a Bausch and Lomb microprojector, and the sections were compared with the Konig and Klippel rat brain atlas [35]

Neurochemical Analysis

Two weeks after surgery, five randomly selected 6-OHDA-treated rats were sacrificed by decapitation and caudate tissue was rapidly dissected over ice using a blunt dissection technique which spared the nucleus accumbens (the anterior commissure was visualized medially) The tissue was stored at −80° until assayed Assays were carried out using tissue from damaged and non-damaged sides of the brain

Caudates were thawed on ice, hand homogenized teflon to glass in 500 μl of pH 7 7 Tris buffer (with 0 1% ascorbate) One hundred μl of caudate was combined with 200 μl of 0 2 N H_3PO_4 containing 10 ng 3,4-dihydroxybenzylamine (DHBA Sigma) as internal standard in 1 5 ml plastic centrifuge tubes One ml of 0 5 M Tris buffer (pH 8 6) and 10 mg Al_2O_3 were added and the suspension shaken intermittently on ice for 10 min to adsorb the catechols Following three rinses with distilled water, catechols were eluted by intermit-

TABLE 1

LATENCY (SECS) TO REMOVE BILATERALLY PLACED ADHESIVE STIMULI FROM SNOUT

	Ipsilateral	Contralateral
6-OHDA	10 30 ± 3 0*	21 6 ± 10 1
Nigra/Electrolytic	17 28 ± 15 2*	55 2 ± 31 7
Sham Control	22 10 ± 13 9	15 40 ± 7 5

*Significantly different from Contralateral

TABLE 2

LATENCY (SECS) TO REMOVE BILATERALLY PLACED ADHESIVE STIMULI FROM RADIAL ASPECT OF FORELIMBS (DERMATOMES C_6–C_7)

	Ipsilateral	Contralateral
6-OHDA	9 59 ± 1 0*†	118 62 ± 28 3†
Nigra/Electrolytic	156 80 ± 57 8*	278 33 ± 21 7
Sham Control	40 83 ± 19 8	37 17 ± 16 2

*Significantly different from Contralateral
†Significantly different from Sham Controls

TABLE 3

LATENCY (SECS) TO REMOVE BILATERALLY PLACED ADHESIVE STIMULI FROM ULNAR ASPECT OF FORELIMBS (DERMATOMES C_8–T_1)

	Ipsilateral	Contralateral
6-OHDA	12 03 ± 2 4*†	92 45 ± 30 1
Sham Control	110 51 ± 42 6	80 03 ± 37 3

*Significantly different from Contralateral
†Significantly different from Sham Controls

tent shaking for 10 min with 75 µl of 0 2 N H_3PO_4 Typical recoveries of extracted standards were >50% Twenty µl portions of the catechol-containing acid were injected into a high performance liquid chromatography (HPLC) system (reverse phase with electrochemical detection) The chromatographic system consisted of a Model 100A metering pump (Beckman Ins), a Model 210 sample injector valve (Beckman Ins) and a reverse-phase 5 µ ultrasphere C18 column (0 46×25 cm) The column eluant was monitored with a commercial amperometric detector Model LC4A (Bioanalytical Systems) with detector potential set at +0 9 V A 4 cm precolumn packed with 5 µ C18 packing protected the analytical column Mobile phase flow rate was 1 3 ml/min The LC4A detector (sensitivity routinely set at 2–10 nA) was connected to a C-R1A data processor (Shimadzu) for initial data analysis

The mobile phase consisted of 0 083 M NaH_2PO_4 H_2O, 0 083 mM Na_2EDTA, 0 1 mM sodium octyl sulfonate, and 8 3% HPLC grade methanol With a 1 3 ml/min flow rate retention times were approximately as follows DHBA=5 9 min, and dopamine=9 0 min (cf , [28, 67])

RESULTS

General Movement Asymmetries

MFB/6-OHDA 6-OHDA-treated animals selected for initial analysis included only those which showed ipsilateral biases in all of the standard tests of asymmetry Data from seven rats having 6-OHDA infusions into the right MFB, and eight rats having lesions in the left MFB were analyzed

Von Frey hair tests These animals readily oriented with their head and body toward the locus of the light tactile stimulation of the snout or forequarters when the Von Frey hair stimulus was on the side of the body ipsilateral to the lesion When the Von Frey hair stimulus was presented contralaterally, no orienting, or less reliable orienting, occurred When the stimuli were presented bilaterally, head movements were elicited toward the ipsilateral side most frequently

Negative geotaxis When they were placed head-down on a 45° slope, they always turned 180° in the ipsilateral direction When placed ipsilateral side-down, they often turned 270° in the ipsilateral direction When placed contralateral side-down, they turned 90° in the ipsilateral direction When the animals were suspended by the base of the tail, they twisted or turned upwards ipsilaterally, or rotated about their longitudinal axis such that the dorsal aspect of their forequarters moved ipsilaterally

Bracing When pushed sideways along the ground in the contralateral direction, they showed exaggerated bracing reactions with the limbs When pushed ipsilaterally, the response varied from stepping only, to slight bracing mixed with stepping reactions of the forelimb (a normal reaction)

Circling movements and turns in the alley occurred ipsilateral to the side of 6-OHDA infusion

Nigra/electrolytic In contrast, the nigra-electrolytic-lesioned animals showed complete, and even more exaggerated, asymmetry in the opposite (contralateral) direction in the circling task, the negative geotaxis tasks, and the alley task It is important to note that no directed responses to Von Frey hairs were obtained in these animals However, the animals appeared to react by increasing their rate of tight contralateral circling, especially if contacted on the side ipsilateral to the lesion

In a follow-up test, the wall of the alley facing the body side concave to the posture of circling was removed In this situation, the nigra-lesioned rats lost their concave posture and walked forward along the other wall, as if guided by the whiskers ipsilateral to the lesion In contrast, the 6-OHDA-treated rats retained their concave bias when the wall facing the body side concave to the posture of circling was removed, which meant that they turned away from the remaining wall Thus, these animals, too, appeared to be guided by their ipsilateral whiskers If allowed to continue, they circled around until they came in contact with the remaining wall, whereupon they reared or walked forward along the wall

Adhesive Removal

MFB/6-OHDA The 6-OHDA-treated animals removed the ipsilateral stimuli consistently sooner than the contralateral stimuli

As shown in Table 1, the rectangular adhesive paper, which was applied bilaterally to the snout, was removed significantly sooner from the ipsilateral side, $\chi^2(1)=7 4$,

$p<0 01$ The stimulus-directed movement involved one or both forepaws, which rapidly brushed downward first on the ipsilateral stimulus During these movements postural asymmetry was biased about 10° in the ipsilateral direction Control animals failed to show significant preference in the order in which the stimuli were removed $\chi^2(1)=0 4$, n s

Removal of the "dot" stimuli from the ipsilateral forepaw occurred sooner than removal of the dot stimuli from the contralateral forepaw, radial (inward) wrists $\chi^2(1)=8 07$, $p<0 01$, ulnar (outward) wrists $\chi^2(1)=15 0$, $p<0 01$, as shown in Tables 2 and 3 Prior to removal of the stimulus, the 6-OHDA-treated rats typically contacted the ipsilateral forepaw before contacting the contralateral paw (radial wrists 6 3±0 7 sec versus 23 1±7 2 sec, $\chi^2(1)=5 4$, $p<0 05$, ulnar wrists 5 2±0 8 sec versus 15 5±2 1 sec, $\chi^2(1)=11 27$, $p<0 01$), as shown in Table 2 Ipsilateral movements typically involved a head turn lateral and ventral toward the stimulus, and then an upward pulling of the dot with the teeth (This movement was also typical of control animals when removing dots from either paw) Contralateral movements typically involved moving the forepaw forward toward the mouth, such that contralateral head movements were minimal

Rapid paw shaking reactions to the adhesive pieces were observed in all rats This occurred more frequently in the contralateral forepaws According to others [66], cats with spinal transections display paw shaking readily with low stimulation of cutaneous afferents In several animals, the contralateral stimulus elicited bilateral paw shakes and face washing movements that led to a full grooming sequence before the animal removed the stimulus

The latency to contact, and latency to remove, the ipsilateral stimuli (for both radial and ulnar dermatomes) were significantly shorter than the latency to contact ($p<0 01$) and the latency to remove ($p<0 01$) the ipsilateral stimuli in control animals Thus, there was 6-OHDA-induced hyperreactivity on the ipsilateral side The latency to contact, and the latency to remove, the contralateral radial stimuli were significantly longer than the latency to contact ($p<0 01$) and the latency to remove ($p<0 01$), the contralateral radial stimuli in control animals (hyporeactivity)

Within control animals, there were no significant asymmetrical preferences for removal of the dots (see Tables 2 and 3) Control animals showed no bias in contacting the forepaw stimuli (radial wrists 9 9±3 4 sec versus 30 7±15 9 sec, $\chi^2(1)=0$, n s , ulnar wrists 22 6±10 4 sec versus 16 4±1 9 sec, binomial X=3, n=9, n s)

In 6-OHDA-treated animals, an ipsilateral bias in hindpaw contact (Means±S E =124 1±52 7 sec versus 367 2±69 0 sec) approached, but did not achieve, significance, $\chi^2(1)=3 77$, $p>0 05$ Some 6-OHDA-treated rats failed to remove the stimuli from either hindpaw, although in all cases during locomotion there occurred abnormal backward shaking and extension movements and increased swing phase in the taped limb However, those animals that did remove the stimuli removed the ipsilateral ones first (binomial X=1, n=9, $p<0 05$) The ipsilateral, stimulus-directed movements involved clear head and body turns toward the hindlimb Contralateral movements often consisted of a midline ventral tuck, along with attempts to move the hindlimb forward toward the mouth such that only minimal or brief contralateral turns were observed

Control animals showed no preference in the order of contact of the hindpaw (ipsilateral=231 2±82 5 sec versus 210 2±70 3 sec, binomial X=4, n=9, n s) or removal of the hindpaw stimuli (ipsilateral=361 7±81 3 sec versus 418 7±76 8 sec, binomial X=2, n=6, n s)

The Wilcoxon matched-pairs signed-ranks test was used to evaluate the differences in time required for the animal to remove the stimuli after an initial mouth contact of either paw 6-OHDA-treated rats showed significantly faster removal of the stimuli on the forepaws ipsilateral to their lesions (radial wrists $p<0 01$, ulnar wrists $p<0 01$) Removal of the hindpaw label was also faster on the ipsilateral side than on the contralateral side ($p<0 01$) Similarly, removal of the snout stimuli occurred more rapidly on the side ipsilateral to the lesion ($p<0 05$) Control animals failed to show significant differences in removal time for the forepaws (radial wrists T=18, N=10, n s , ulnar wrists T=6, N=8, n s), the hindpaws (T=14, N=6, n s), or the snout (T=25, N=10, n s)

Thus, it appears that comparative responsiveness to bilateral adhesive stimuli is a reliable measure of asymmetries in brain function in rats It is interesting to note that even rats which showed contralateral neglect during conventional tests of orienting using Von Frey hairs, nevertheless were able to localize (see also [44,70]) and remove stimuli on the contralateral snout and forepaw Neglect of the contralateral hindpaw stimulus did occur, and four rats did not remove the stimuli from either hindpaw in the time allotted

Nigra/electrolytic The behavior of the rats with substantia nigra electrolytic lesions was extremely interesting (see Tables 1 and 2) In contrast to the 6-OHDA-treated rats, all of the rats exhibited non-directed (spontaneous) contralateral circling, contralateral flexion when suspended by the base of the tail, and contralateral postural asymmetry, however, when adhesive stimuli were applied to their forepaws, they removed the stimulus from the ipsilateral side first ($p<0 01$) The latency to first contact the ipsilateral stimulus was faster than that for the contralateral stimulus (61 2±47 8 versus 212 2±53 4 sec, $p<0 01$) Three of the six rats exhibited total neglect of the stimulus on the contralateral forepaw, which never occurred in MFB/6-OHDA-lesioned animals It was striking to observe stimulus-directed reactivity convex to postural orientation simultaneous with neglect of stimuli concave to postural orientation The asymmetry of responsiveness to adhesive stimuli applied to the snout was similar Four of six rats consistently contacted the snout ipsilateral to the lesion first and removed the stimulus from the ipsilateral snout before removing the one on the contralateral snout Two of these rats showed total neglect of the stimulus on the contralateral snout Another rat briefly exhibited ineffective scratching movements at the stimulus using the contralateral hindlimb The two rats with the smallest lesions removed both snout stimuli simultaneously by making bilateral face-wiping movements of the forepaws

All of these rats failed to remove the hindpaw stimuli from either side, although an obvious increase in swing phase of locomotion and backward shaking and extension of the stimulated limb was induced However, the failure to remove the ipsilateral hindlimb stimulus was probably due to their exceptionally strong contralateral circling behavior and complete lack of ipsilateral turning, which could have prevented them from making the required directed movement toward the apparently more "sensitive" ipsilateral side

Neurochemistry and Histology

High performance liquid chromatography revealed that the ipsilateral caudate contained only 28 3% (median) of the dopamine in the contralateral caudate (the greatest relative

TABLE 4

SUMMARY OF STIMULUS-DIRECTED AND STIMULUS-INDEPENDENT MOVEMENT BIASES

	Spontaneous Circling*	Von Frey Orienting†	Adhesive Removal‡
6-OHDA	ipsi	ipsi	ipsi
Nigra/electrolytic	contra	no response	ipsi
Sham Control	no bias	no bias	no bias

*Stimulus-independent head and body turns.

†Stimulus-directed head and body turns.

‡Stimulus-directed adhesive-removing movements.

depletion was 8.6% and the least was 71.1%). Although other neural areas were not assayed, it should be expected that the 6-OHDA injection depleted multiple catecholaminergic terminal fields. Binomial tests indicated significant differences between ipsilateral and contralateral dopamine ($p<0.05$).

Histological examination revealed that the substantia nigra electrolytic lesions were large and involved all or most of the substantia nigra (pars compacta and pars reticulata) and adjacent regions. The size and location of the lesion cavities were similar to those described previously [61]. However, the cell-poor area of chromatolysis often encompassed other important tissue, including the midbrain reticular formation (n=6), the ventral nucleus of the thalamus (n=2), and the lateral aspect of the crus cerebri (n=1). Damage to any one of these areas could well have contributed to the symptom combination of contralateral circling and ipsilateral stimulus-directed adhesive removal bias.

DISCUSSION

The major findings are outlined in Table 4. An ipsilateral bias in stimulus-directed activity was seen in the 6-OHDA-treated rats. Moreover, there was an increase in reactivity on the ipsilateral side relative to sham controls (see also [22,24]). This is reminiscent of the enhanced effects on sensorimotor reactivity of electrical stimulation of the contralateral medial forebrain bundle area [15,65], and it may be related to the sensory-rejection reactions observed in animals with bilateral MFB lesions [60]. Hypersensitivity ipsilateral to destruction of ascending pathways is also consistent with previous behavioral and neurochemical demonstrations of interhemispheric interactions at this and other levels of the neuraxis [11, 39, 49].

Despite a strong "spontaneous" contralateral motor bias involving stimulus-independent movements (i.e., the stimulus is unknown to the experimenter, but is probably self-generated), the rats with electrolytic lesions of the nigra likewise were biased toward the ipsilateral side in a bilateral adhesive-removal test which involved stimulus-directed movements but did not require head turns. Responsiveness to adhesive tactile stimuli appears to be a useful way of dissociating postural/motor asymmetries, in a quantitative way, from certain "sensorimotor" asymmetries in rats.

Thus, the potentially paradoxical finding that contralaterally biased movements occur in rats with substantia nigra lesions (while ipsilaterally biased movements occur in rats with more selective 6-OHDA infusions), makes sense. If the test does not require stimulus-directed large lateral movements of the head, the "sensorimotor" biases are both ipsilateral. This commonality presumably reflects similar asymmetries in ascending catecholaminergic pathways. The contralateral motor bias is not surprising. There are a number of key outposts of the spinal motor system that receive inhibitory input sent caudally from forebrain regions. These structures, including some located in the region of the substantia nigra, soon cross and descend to the spinal cord [39, 53, 63, 77]. Damage to these efferents near the origin may be expected to yield circling effects opposite to those observed after more rostral (presynaptic) damage (i.e., damage to forebrain efferents such as the striato-nigral projections).

In conclusion, we believe that a measure of the direction and degree of circling behavior is far more powerful when it is combined with other measures such as the relatively turn-free adhesive-removal test that separately quantifies tactile-stimulus directed behavior. It remains to be demonstrated that this procedure will prove useful for assessing behavior following alterations in other "sensory-motor" brain areas. However, for now we suggest that the recent attention given to imposed and natural asymmetries in afferent and efferent striatal projections (e.g., [3, 5, 9, 17, 19, 23, 33, 36, 43, 55, 57, 73, 75]) might benefit from a battery of behavioral tests that includes bilateral adhesive paper removal.

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