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Role of Pars Compacta of the Substantia Nigra in Circling Behavior

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HODGE, G. K. AND L. L. BUTCHER. *Role of pars compacta of the substantia nigra in circling behavior.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 695–709, 1979.—The significance of pars compacta of the substantia nigra in the mediation of circling behavior was assessed by placing unilateral radio-frequency lesions in that neural region, in adjoining tegmental areas, or in the median raphe and adjacent reticular formation; additional ablations were made by infusing 8 μg/4 μl 6-hydroxydopamine (6-OHDA) into the ventral mesencephalon. Rotational behavior was recorded in a photocell apparatus during 15 min sessions. Precise and virtually complete lesions of pars compacta, as evidenced by histological and histochemical analyses, resulted in spontaneous contralateral circling for a postoperative period of 5 days. More vigorous and sustained contralateral rotations were observed in animals with lesions in the region of the median raphe. Other thermal or 6-OHDA mesencephalic lesions did not produce spontaneous circling. On postoperative days 9–23, the animals were administered in random sequence d-amphetamine (1.5 and 5.0 mg/kg) and apomorphine (1.0 mg/kg). At least 48 hr separated each drug test. Amphetamine (1) facilitated contralateral turning in animals with damage to the median raphe and adjacent regions but induced ipsilateral circling in animals sustaining variable degrees of pars compacta damage as a result of midbrain 6-OHDA injections, (2) produced ipsilateral turning in rats with lesions involving portions of pars compacta in addition to the medial lemniscus (at 1.5 but not 5.0 mg/kg drug), pars reticulata (at 5.0 but not 1.5 mg/kg drug), or the ventromedial tegmentum and, in some cases, subthalamic regions (at 5.0 but not 1.5 mg/kg drug), and (3) had no effect upon circling in animals with discrete pars compacta lesions in a dose of 1.5 mg/kg but produced ipsiversive circling after 5.0 mg/kg. Apomorphine did not elicit turning in animals with relatively precise lesions of pars compacta but did produce turning in experimental groups that sustained simultaneous damage to pars compacta and additional systems including portions of the ventromedial tegmentum, pars reticulata of the substantia nigra, or medial lemniscus. The contralateral turning induced by apomorphine in rats with 6-OHDA or radio-frequency lesions of the ventral mesencephalon suggests that mechanisms mediating this drug-induced behavior, although previously attributed almost exclusively to dysfunctions in nigral function, probably include systems involving extra-nigral regions as well. Coupling this consideration with the finding that lesions in the median raphe and contiguous areas elicited more intense circling than nigral ablations suggests that the role of pars compacta in rotational behavior may be less significant than previously believed.

Circling Pars compacta of the substantia nigra Dopamine Apomorphine Amphetamine Median raphe

CIRCLING can be produced by intracerebral injection of a variety of chemicals (e.g., carbachol [34], dopamine [48], di-isopropylphosphorofluoridate [49]), as well as by various unilateral brain lesions, including those placed at different locations in the nigrostriatal and other mesencephalo-telencephalic projections, in the frontal cortex, in nucleus centralis superior (median raphe) and contiguous areas, and in the locus coeruleus and adjacent loci (see [24]). Despite the diverse nature of these experimental manipulations, the system whose altered function is thought to contribute most significantly to circling behavior is the nigrostriatal dopamine pathway (for review and critical analysis, see [24]).

The mechanism responsible for these asymmetries in motor behavior has been hypothesized to involve a functional imbalance between the two ipsilaterally projecting dopamine pathways (e.g., see [2, 24]). When the side of the brain containing the more efficacious dopamine system becomes activated, either physiologically or as a result of injections of drugs whose actions are believed to involve presynaptic (e.g., amphetamine) or postsynaptic (e.g., apomorphine) dopamine mechanisms [24], the motor influences of that side dominate so that the animal tends to circle toward the side of the body containing the less efficacious dopamine system. Further arguments that a dopamine pathway is the primary system involved in circling have been advanced by Glick *et al.* [25]. These investigators presented evidence that rotation in normal animals is correlated with small, naturally occurring imbalances in striatal dopamine—but not acetylcholine—concentrations. In addition, systemic injection of d-amphetamine can elicit circling contralateral to the side of higher dopamine content [25]. Predicated on the belief that circling behavior is mediated by dopaminergic systems, data have been presented suggesting that cholinergic [11, 16, 30, 33], serotonergic [13,14], noradrenergic [28,37], and GABAergic [42] mechanisms interact

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with the nigrostriatal system to modify circling behavior.

Not all striatal dopamine derives from *nigral* sources, however. Lesions restricted primarily to pas compacta of the substantia nigra, the A9 dopamine somata of Dahlström and Fuxe [17], reduce striatal dopamine to only approximately 40% of normal whereas combined lesions of pars compacta and the ventromedial mesencephalic tegmentum, including the A10 dopamine group [17], decrease caudate-putamen dopamine to subdetectable levels [22]. Furthermore, large lesions of the caudate-putamen nucleus, in addition to producing retrograde loss of pars compacta somata, produce retrograde degeneration of a small but definite number of neurons in the ventromedial mesencephalic tegmentum [10]. There is also evidence that A8 dopamine somata project to the caudate-putamen complex [43]. And, finally, Fallon and Moore [21] have presented data that question the anatomical validity of strict A8-A10 somata delimitations and similar rigid delineations of their respective projections, particularly as proposed by Ungerstedt [43].

Although procedures that affect dopaminergic systems of the midbrain undoubtedly affect circling, it is unwarranted, based upon available data, to attribute this effect exclusively to the nigrostriatal system: the striatum receives dopamine afferents from neuronal somata located outside pars compacta [10] and pars compacta apparently projects to areas in addition to the striatum [21].

An additional caveat is that most, if not all, previously published work where asymmetries were induced by lesions have not been sufficiently precise to demonstrate a primary role for nigrostriatal dopamine in mediating rotational behavior. First, the electrolytic lesions in studies of circling invariably have involved areas of the brain containing systems distinct from components of the nigrostriatal dopamine pathway (e.g., compare [2,3] with [32]). And second, the cytotoxin 6-hydroxydopamine (6-OHDA) in the amount most often used to induce turning, 8 μg in 3–4 μl vehicle (e.g., see [38,47]), has been reported to produce significant non-selective destruction of neural tissue [1, 7, 9, 27, 36, 41]. Furthermore, even if 6-OHDA selectively destroyed dopamine somata, it would seemingly show no discrimination between dopamine cell bodies of pars compacta and those of immediately surrounding areas.

Considering these data collectively, we contend that the importance of the nigrostriatal dopamine system, specifically those fibers arising from pars compacta, has been overstated in previous papers and is in need of re-evaluation. Our approach has been to produce radio-frequency lesions restricted virtually exclusively to pars compacta of the substantia nigra. Since 80–90% of all somata in that nigral subdivision appear to contain dopamine (Fuxe, personal communication), such ablations should involve nigral dopamine mechanisms importantly, but, of course, not exclusively. Nonetheless, we believe our ablations are among the most selective yet produced in the pars compacta, and as such, should provide useful information about the role of that somata aggregation in circling, as well as perhaps providing a basis for the development of even more selective lesion procedures, including those involving nigral neurons possessing particular neurochemical characteristics. The findings from these lesion experiments are compared with the effects on turning of mesencephalic 6-OHDA lesions and radio-frequency lesions in the region of the median raphe nucleus, which contains B8 serotonin somata (terminology of Dahlström and Fuxe [17]). The effects on turning of two drugs affecting dopamine mechanisms, amphetamine and apomorphine, are also reported.

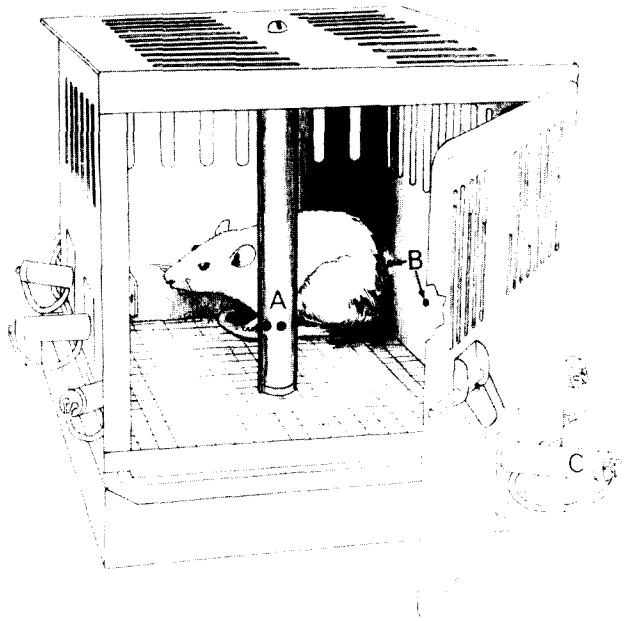

FIG. 1. Apparatus for assessment of circling. Light is emitted from the central column (A), passes through the apertures (B), and falls on the photocells (C). The light source is a 6-volt tungsten bulb. Infrared filters cover the apertures (B).

METHOD

Animals

Adult female rats of the Swiss-Webster strain (Simonsen Laboratories; Gilroy, CA) were individually housed in sheet-metal cages under conditions of constant temperature (22°C), relative humidity (50%), and illumination (24 hr light). At the time of surgery, the animals weighed between 280 and 360 grams.

Surgical Procedures

The rats were anesthetized with sodium methohexital (Sodium Brevital®, 50 mg/kg). Their heads were shaved and then mounted by use of ear plugs in a Trent-Wells stereotaxic instrument (Trent-Wells, Inc.; South Gate, CA). The upper incisor bar was located 2.8 mm below the interaural line; this provided an angle of 5° between the horizontal plane and a plane determined by the interaural line and a parallel line passing immediately behind the upper incisors (see [31], p. 3).

The electrode used in making radio-frequency lesions of pars compacta of the substantia nigra was a teflon-coated stainless steel wire (coated diameter: 0.28 mm; uncoated diameter: 0.20 mm). The teflon at the tip was removed exposing 1.5 mm of the stainless steel wire. Taking advantage of the anatomic orientation of pars compacta to make selective lesions in that neural region, we introduced the electrode into the brain at an angle of 45° from the horizontal. Two such insertions were made. The stereotaxic coordinates (bregma zero) were: posterior, 5.5 mm and 6.4 mm; lateral, 9.7 mm and 9.5 mm; diagonal from the dura mater, 7.5 mm. At each electrode placement, the current was increased

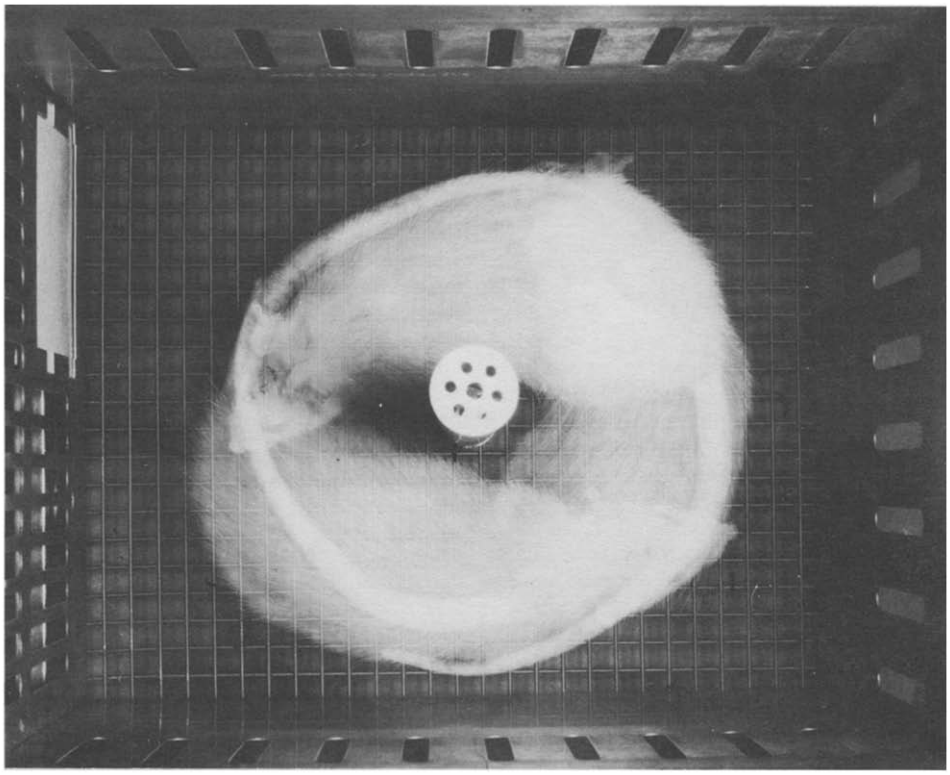

FIG. 2. Stroboscopic photograph showing circling of a rat with an asymmetric lesion in the median raphe nucleus and adjacent reticular formation. When the animal completes a noninterrupted 360° turn around the central column one rotation is counted.

slowly over a 60 sec interval from zero to 14 mA and maintained at this level an additional 60 sec. A rectal ground was used.

Radio-frequency lesions of the median raphe area were made with a stainless steel electrode 0.7 mm in diameter that was teflon-coated except for 1.5 mm at the tip. It was inserted once into the medial raphe area at an angle of 75° from the horizontal to avoid perforation of the saggital sinus. The coordinates (bregma zero) were: posterior, 5.9 mm; lateral, 2.0 mm; diagonal from the dura mater, 8.6 mm. Other parameters were identical to pars compacta procedures.

Sham-operated controls were treated exactly like the radio-frequency lesion animals except that no current was passed.

Midbrain 6-OHDA lesions were made by intracerebrally infusing 8 μg of the cytotoxin in 4 μl of 0.9% saline containing 0.2 μg/μl ascorbic acid. Intranigral injection of this saline-ascorbic acid vehicle has been reported to be without effect on circling [47] and brain catecholamine histochemistry [47]. A stainless steel cannula (outer diameter: 0.46 mm) was used. The rate of infusion was 0.5 μg/min. Infusion was begun 1 min after the cannula was inserted at the following stereotaxic coordinates (bregma zero): posterior, 5.7 mm; lateral, 1.8 mm; vertical from the dura mater, 6.9 mm. After termination of flow, the cannula remained in place for an additional 3 min before being slowly withdrawn.

Behavioral Procedures

Spontaneous rotational behavior was recorded 6–10 and 14–18 hr after surgery and for the subsequent 7 postoperative days. Each rat was tested individually. The experimental chamber in which circling behavior was recorded was identical to the rat's home cage except for the modifications illustrated in Fig. 1. A column containing an infrared light source was located in the center of the cage (Fig. 1,A). Eight apertures, each 5 mm in diameter, were located 4 cm from the floor around the periphery of the apparatus (Fig. 1,B). Beams of light emanating from the column passed through the apertures and fell on photocells housed in tubes attached to the outside of the apparatus (Fig. 1,C). An Intellec 8080 microprocessor (Intel Corporation; Santa Clara CA) was used to collect the data. The computer program required that a beam be interrupted for at least 0.5 sec before a beam-breaking was counted. This minimized a number of artifactual counts such as those due to the animal flicking its tail across a beam. A single rotation and its direction, either clockwise or counterclockwise, was counted only if the animal traversed an uninterrupted 360° turn around the central column (Fig. 1,A; Fig. 2). As long as the rat continued to circle the column unidirectionally and did not recross a previously encountered beam, a rotation was scored, but if the animal hesitated, reversed direction, and recrossed a previously interrupted beam, then the program reset, requiring the rat to traverse a new 360° rotation from that point. Over the 15 min testing period normal animals would satisfy these criteria infrequently, their activity being more randomly distributed about the cage (Fig. 3). In none of the experimental or control groups did any of the animals merely sit in the corner of the chamber and turn; all rats locomoted in relation to the central column.

Pharmacologic Procedures

On postoperative days 9–23, d-amphetamine sulfate (1.5 and 5.0 mg/kg as salt; K & K Laboratories; Plainview NY) and apomorphine-HCl (1.0 mg/kg: Eli Lilly and Co.: Indianapolis IN) were on separate occasions administered (1 ml/kg, intraperitoneally) once to each rat in random order. The vehicle was 0.9% saline. Following injections, the animals were immediately placed within the recording apparatuses (Fig. 1), and ensuing rotations were counted in 15 min intervals over a 90 min period. At least 48 hr elapsed between successive drug injections.

Histologic and Histochemical Procedures

Thionin staining and treatment of lesion data. Lesion placements in all animals in the behavioral experiments were evaluated in thionin-stained brain sections. The rats were anesthetized with sodium pentobarbital and were sacrificed by cardiac perfusion with 20 ml 0.9% saline followed by 20 ml buffered Formalin (pH = 7). The brains were removed and stored in buffered Formalin for at least 48 hr. These then were transferred to beakers containing a 30% sucrose solution for an additional 48 hr. The brains were blocked in the planes of orientation of König and Klippel [31] and were sectioned in 80 μm intervals. The sections then were mounted in serial order on glass slides, dried, stained with thionin as described in Skinner [40], and cover-slipped.

The histologic data were evaluated in the following manner: the brain slides from each experimental animal were placed in a projecting microscope. Tracings of every second or third 80 μm section were then made through the different levels of the lesion's extent. The series of tracings then were placed together to form a rostrocaudal or caudorostral display of the lesion. The neuroanatomic terminology and format of these displays are shown for nonlesion animals in Fig. 4 and 5. The maximum extent of the lesions as indicated by the presence of cavitation and gliosis is represented by solid black areas (Fig. 6,C; compare with Fig. 6,A and with Fig. 6,B and D), although in some cases this was no more than a thin line (e.g., see ROTA 52 in Fig. 7).

Catecholamines and acetylcholinesterase. In addition to the behavioral studies, separate histochemical experiments were performed to assess the effects of the experimental lesions on forebrain catecholamine fluorescence. In these latter experiments unilateral radio-frequency ablations were attempted in the substantia nigra or median raphe area according to procedures detailed earlier in this report. Two weeks later the animals were sacrificed by decapitation. The brains were removed from the cranial cavity and divided into two pieces with a razor blade. The cut was made at the level of the mid-hypothalamus in the coronal plane of König and Klippel [31]. The anterior portion containing forebrain projection areas of the substantia nigra, pars compacta and other catecholamine somata was processed for catecholamines according to the glyoxylic acid method of de la Torre and Surgeon [18]. The posterior portion containing the lesion was stained for acetylcholinesterase according to the method of Karnovsky and Roots [29] as slightly modified by Butcher *et al.* [9]. The substantia nigra is demonstrated especially well with acetylcholinesterase histochemistry (e.g., see [8]).

Evaluations of fluorescence decrements or increments in histochemical studies cannot provide precise quantitative data. In the present studies we assessed catecholamines qualitatively by noting increases or decreases in fluorescence intensity. In the forebrain areas we examined, such

FIG. 3. Stroboscopic photograph showing absence of circling in a nonlesioned rat.

intensity changes could reflect alterations in the number of terminals and/or changes in catecholamine content in individual terminals. Since we employed relatively thick tissue sections (30 μm), evaluation of number of individual catecholamine terminals was not possible in the nucleus accumbens, caudate-putamen complex, and olfactory tubercle, in which terminal density is so high as to preclude observation of separate neuronal endings. Accordingly, in these structures, visual estimates of overall intensity only were made (e.g., see Dahlström and Fuxe [17] and Ungerstedt [43]). In forebrain regions where individual terminals could be discerned with the tissue thicknesses used (e.g., nucleus interstitialis of the stria terminalis), visual estimates of number of terminals were made. Fluorescence intensity estimates of individual terminals was not attempted systematically.

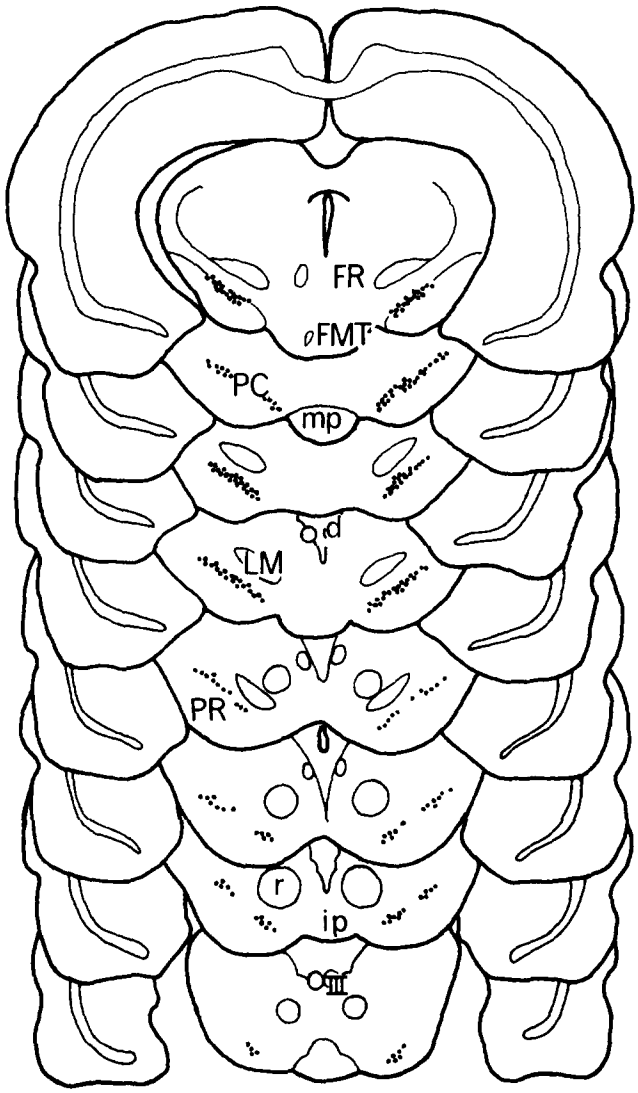

FIG. 4. Neuroanatomic terminology and format of histologic display for lesions placed in the ventral mesencephalon (see Fig. 7–10, 12). Tracings of brain sections are arranged rostrocaudally. A nonlesioned brain is shown. Black dots represent neuronal somata of pars compacta (PC) of the substantia nigra. FR, fasciculus retroflexus; FMT, mamillothalamic tract; mp, posterior mammillary nucleus; d, nucleus Darkschewitsch; LM, medial lemniscus; PR, pars reticulata, substantia nigra; r, red nucleus; ip, interpeduncular nucleus; III, occulomotor nucleus.

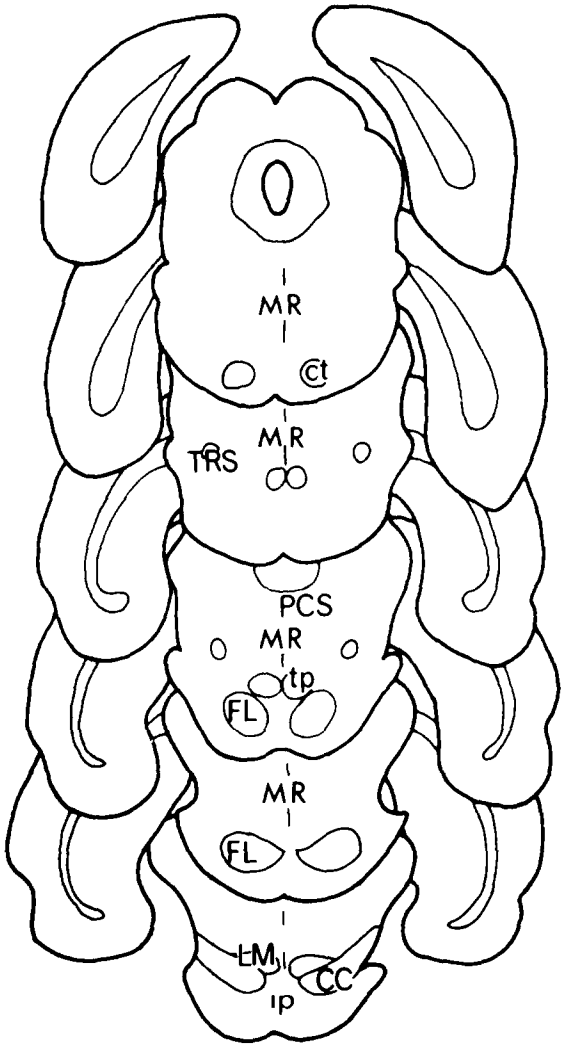

FIG. 5. Neuroanatomic terminology and format of histologic display for lesions placed in the median raphe and adjacent regions (Fig. 11). Tracings of brain sections are arranged caudorostrally. A nonlesioned brain is shown. MR, median raphe nucleus; ct, nucleus corporis trapezoidei; TRS, rubrospinal tract; PCS, superior cerebellar peduncle; tp, nucleus tegmenti pontis; FL, fasciculus longitudinalis; LM, medial lemniscus; CC, crus cerebri; ip, interpeduncular nucleus.

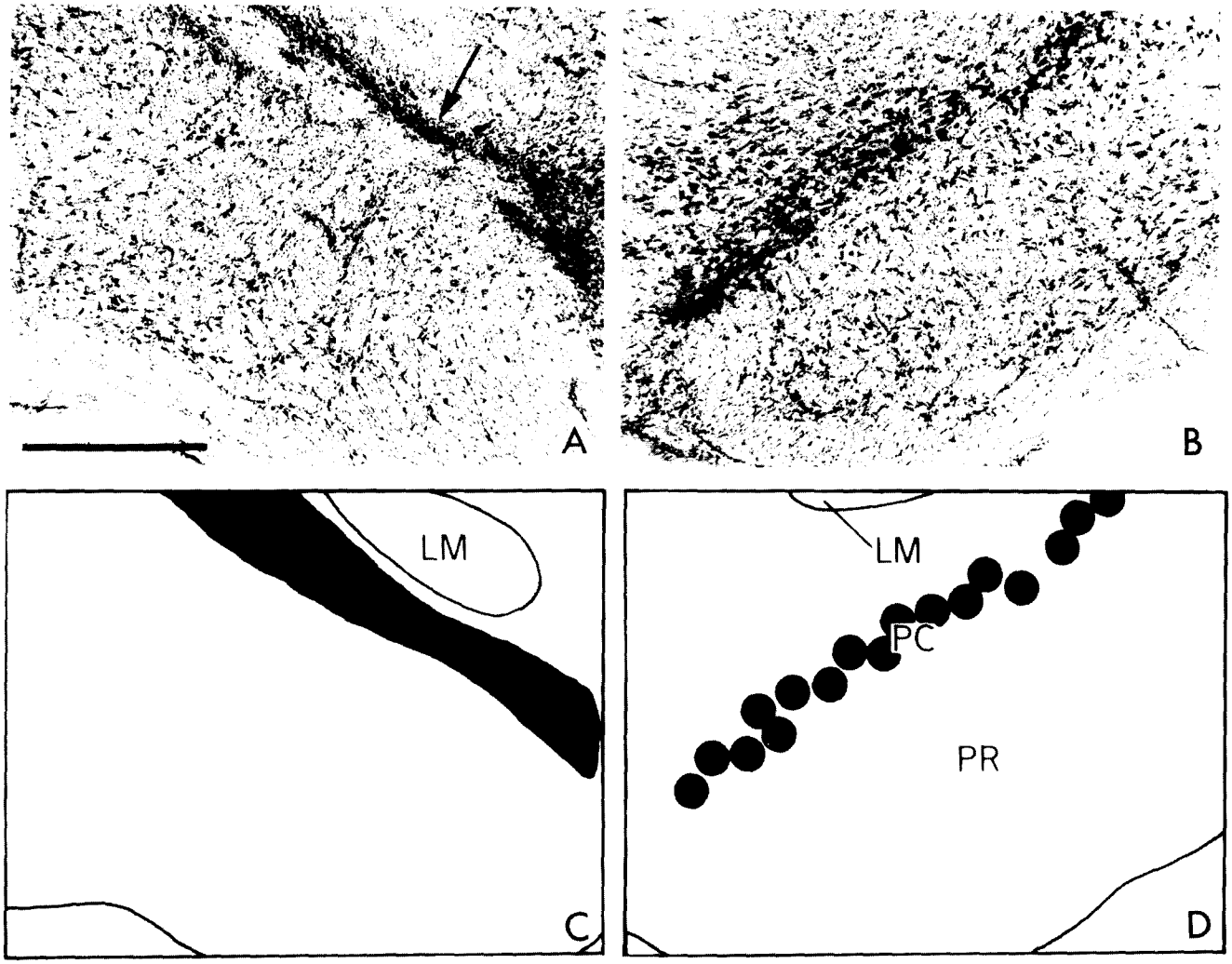

FIG. 6. Radio-frequency lesion of pars compacta (PC) of substantia nigra (A) and schematic representation of that lesion (C). Non-lesioned side and corresponding schematic representation are shown in B and D, respectively. Thionin stain in A and B. Rat lesioned with 14 ma current and sacrificed 8 weeks thereafter. Section thickness in A and B is 80 μm. Arrow in A points to a layer of glial cells that is collapsing toward the ventral edge of the lesion. Solid black region in C delimits the area of tissue necrosis; this representation also applies to tissue necrosis in Fig. 7–12. Clusters of pars compacta somata are indicated by solid black circles in D; compare with B. LM, medial lemniscus; PR, pars reticulata, substantia nigra. Scale, 500 μm.

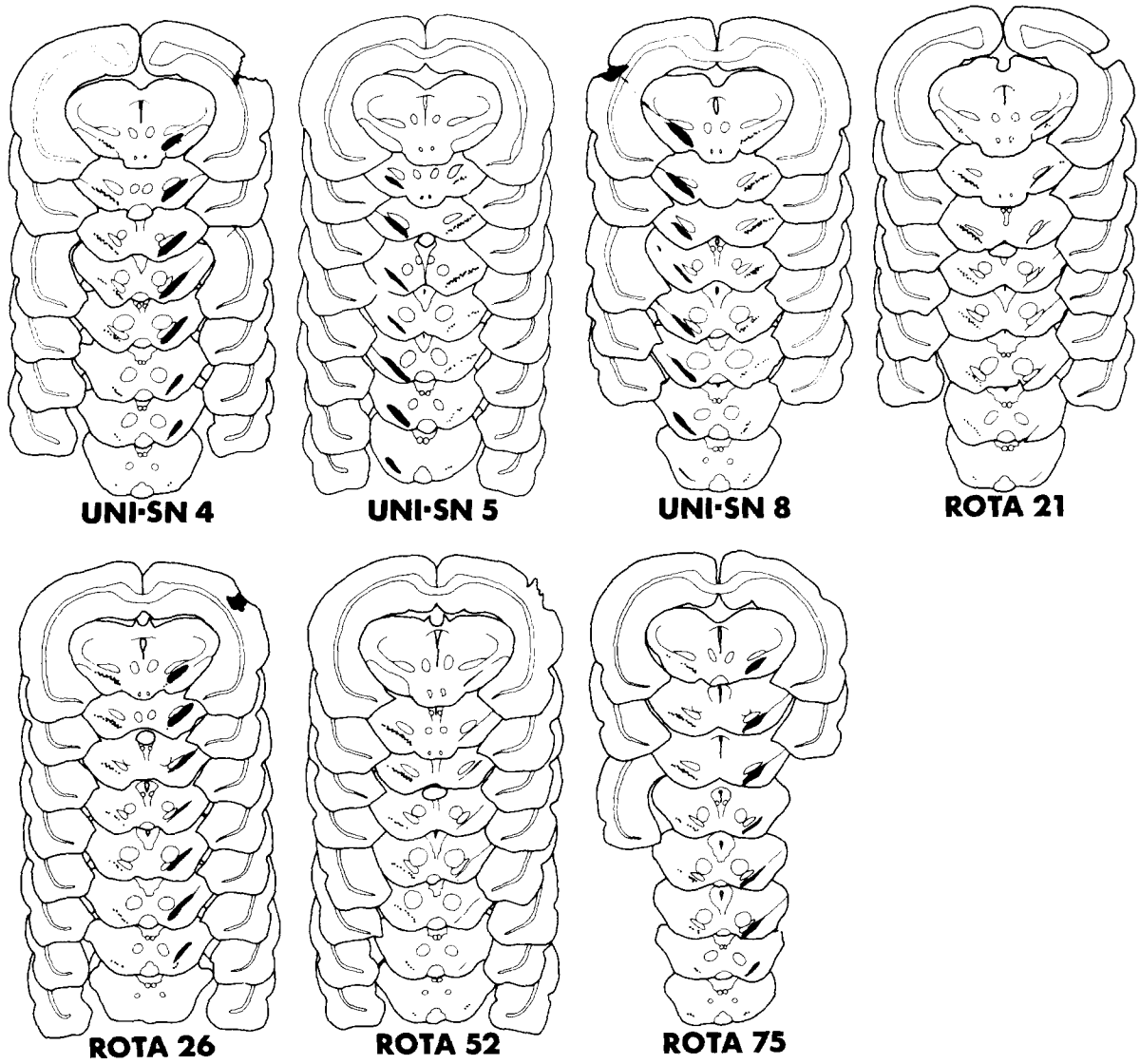

FIG. 7. Radio-frequency lesions representing the range of neuropathologic involvement in group PC. Two rats' histologies are not shown. UNI-SN 4, 5, and 8 were sacrificed 10 days after lesion placement; ROTA 26 and 75 at 3 weeks; ROTA 21 and 52 at 24 weeks. For further explanation see text and legends of Fig. 4 and 6.

RESULTS

Composition of Experimental Groups

On the basis of experimental treatment and histologic results, the animals were grouped into one of the following 8 categories: (A) non-lesion controls (n=14, Fig. 4-5), (B) operated controls (n=15), (C) radio-frequency lesions producing virtually complete loss of pars compacta cell bodies in the substantia nigra with minimal extra-nigral damage (group PC, n=9, Fig. 7), (D) radio-frequency lesions involving variable loss of somata in pars compacta and extending into the ventromedial tegmentum, including, in some cases, subthalamic regions (group PC-VMT, n=4, Fig. 8), (E) radio-frequency lesions involving variable loss of somata in pars compacta and portions of the medial lemniscus (group PC-ML, n=4, Fig. 9), (F) radio-frequency lesions involving variable loss of somata in pars compacta and portions of pars reticulata of the substantia nigra (group PC-PR, n=4, Fig. 10), (G) radio-frequency lesions of the median raphe and contiguous areas (group MR, n=10, Fig. 11) and (H) 6-OHDA lesions in the ventral mesencephalon (group 6-OHDA, n=7, Fig. 12).

Histologic and Histochemical Evaluations

Representations of lesion extents are shown in Fig. 7–12. The most selective lesions of pars compacta in the current study were those in group PC animals (Fig. 7). Although damage was produced by the passage of the lesioning probe through brain regions dorsolateral to the substantia nigra and to variable portions of the area between pars compacta and the medial lemniscus, this neuropathology appeared slight in group PC. Indeed, in sham-operated animals there was no histochemically detectable effect on forebrain catechol-

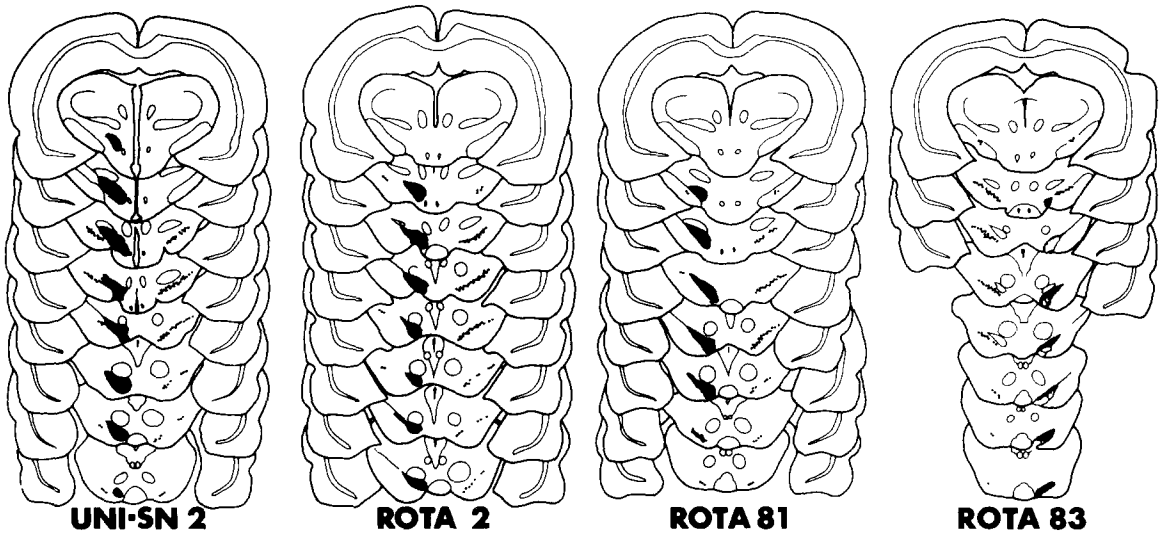

FIG. 8. Radio-frequency lesions representing the range of neuropathologic involvement in group PC-VMT. UNI-SN 2 was sacrificed 10 days after lesion placement; ROTA 81 and 83 at 3 weeks; and ROTA 2 at 26 weeks. For further explanation see text and legends of Fig. 4 and 6.

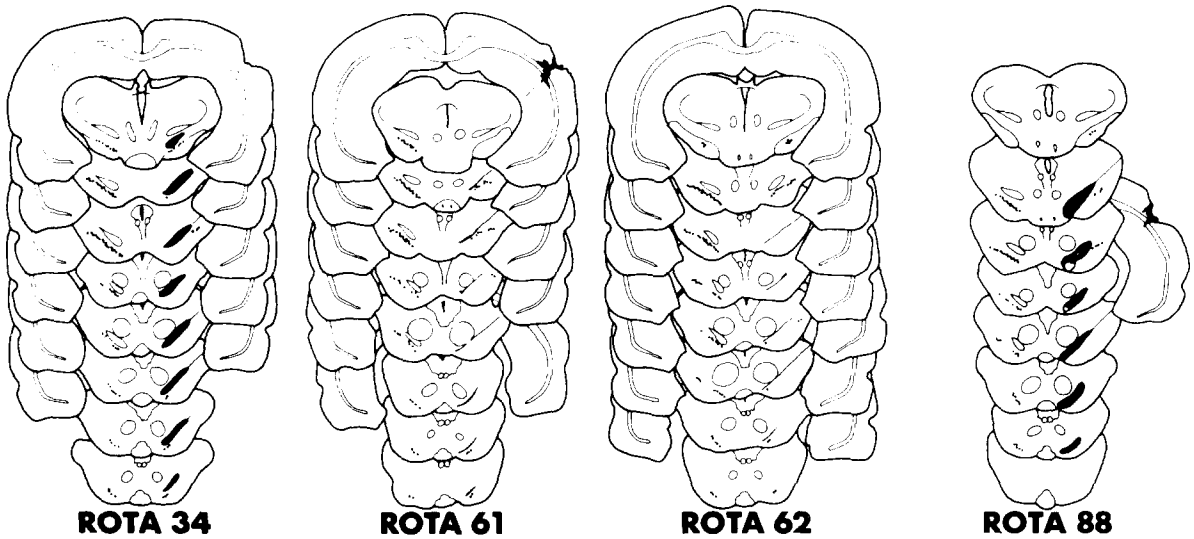

FIG. 9. Radio-frequency lesions representing the range of neuropathologic involvement in group PC-ML. ROTA 34 and 88 were sacrificed 3 weeks after lesion placement; ROTA 61 and 62 at 24 weeks. For further explanation see text and legends of Fig. 4 and 6.

amines. In some group PC rats a few cell bodies remained intact at caudal-most levels of the compacta (Fig. 7).

Ablations restricted almost exclusively to pars compacta produced a severe but not complete loss of fluorescence in the caudate-putamen complex (Fig. 13); this was the most significant and consistent histochemical finding in animals sustaining such lesions. The reduction was greatest at caudal and intermediate levels of the nucleus. Rostral portions of the striatum showed definite but less severe fluorescence decrements. Fluorescence intensity in the nucleus accumbens and olfactory tubercle, two major projection areas of the A10 somata group in the ventromedial mesencephalic tegmentum, was unaffected or only slightly decreased as assessed histochemically. Catecholamine fibers and terminals in the septum and amygdala showed only slight, if any, decreases in number of fluorescent terminals. Slight to moderate loss of dopamine terminals was consistently observed, however, in the interstitial nucleus of the stria terminalis.

Decreases in striatal fluorescent intensity of animals sustaining lesions similar to those of experimental groups PC-ML and PC-PR (Fig. 9, 10) were highly correlated with the amount of pars compacta involvement, which ranged from moderate to severe. If cell bodies of pars compacta remained after lesions in the PC-ML or PC-PR groups, these were located primarily at rostral levels (Fig. 9, 10). Little or no loss of telencephalic catecholamine fluorescence (overall intensity or number of terminals) was observed after ablations similar to those shown in Fig. 11 (group MR), except when they affected known catecholamine systems originating in or traversing the metencephalon and mesencephalon.

Radio-frequency lesions that extended into the ventromedial tegmentum and included in some instances the subthalamus, as well as involving a substantial number of pars compacta neurons as observed in group PC-VMT (Fig. 8), produced a marked decrease in overall fluorescence in the caudate-putamen complex. Furthermore, there was considerable loss of overall fluorescence in nucleus accumbens and the olfactory tubercle, as well as a marked reduction in number of terminals in the septum, interstitial nucleus of the stria terminalis, and the amygdala. In our hands, the glyoxylic acid protocol of de la Torre and Surgeon [18] did not permit reliable evaluation of catecholamine loss in the cortex.

Ablations induced by 6-OHDA were characterized by significant non-selective destruction of neural tissue (Fig. 12), a result in agreement with numerous previous reports [1, 7, 9, 27, 36, 41]. Although such lesions involved most pars compacta, they also typically included the medial lemniscus and parts of the red nucleus and/or adjacent lateral tegmental regions (Fig. 12).

Behavioral Observations

Spontaneous rotational activity. Animals with asymmetric lesions in the region of the median raphe (group MR) or preferentially in pars compacta (group PC) exhibited circling contralateral to the side of the ablation (Fig. 2, 14). Although turning was considerably more marked in the MR group than in the PC animals, the maximum number of rotations occurred 1–2 days after surgery in both groups. Significant ipsilateral turning also was seen in animals in group MR, but this was not as pronounced as their tendency to circle contralaterally to the side containing the major extent of damage (Fig. 14). By the end of the 7 day testing period, rats in group MR continued to turn significantly to the contralateral side, whereas circling in group PC animals did not differ from controls (Fig. 14).

In addition to quantitative differences between the circling profiles of the MR and PC groups, qualitative differences were noted as well. Rotations by some rats in the MR group consisted of bursts of movement from one corner of the testing chamber to the diagonally opposed corner, the animals describing an elliptical pattern around the central column. The rats would sometimes pause in the corner before resuming their progression, which, when resumed, often appeared as if they were being compelled to move (cf. [28]). In contrast, rotational behavior manifested by rats in the PC group consisted of the animals walking around the central

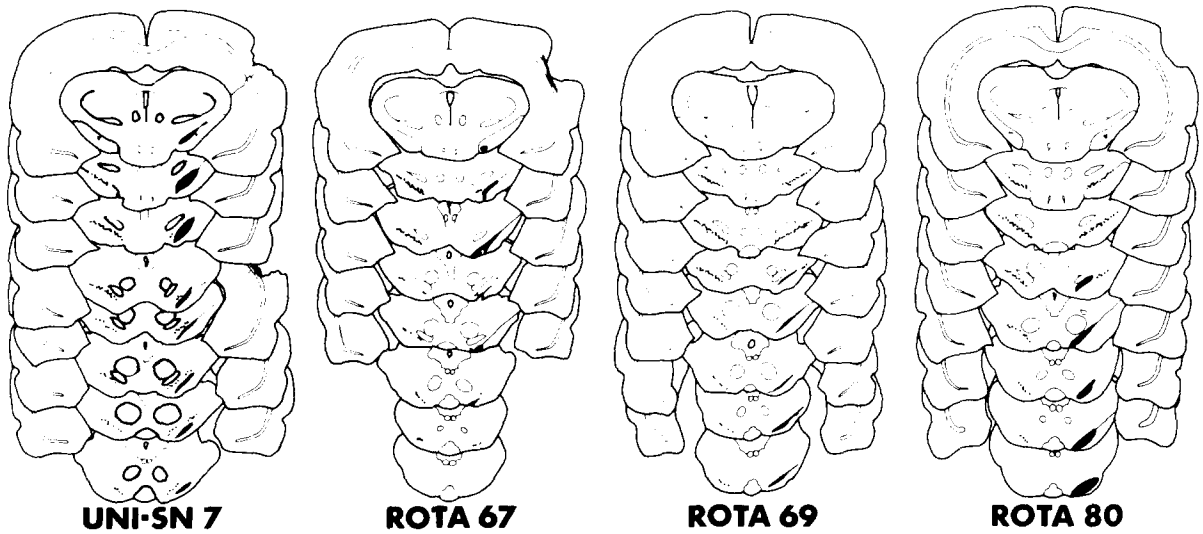

FIG. 10. Radio-frequency lesions representing the range of neuropathologic involvement in group PC-PR. UNI-SN 7 was sacrificed 9 days after lesion placement; ROTA 67, 69, and 80 at 3 weeks. For further explanation see text and legends of Fig. 4 and 6.

column in a slow but deliberate manner. They would stop to groom, to rear, and to look about, but eventually they would continue their progression around the column. Except for this asymmetric predisposition in locomotor activity, these animals appeared normal as assessed by gross observation.

No other experimental groups in the current study circled spontaneously in the testing chamber (Fig. 14), and the quantitative data obtained in the photocell apparatus in all experimental groups were corroborated by separate observations of the animal's behavior when they were placed on a large flat surface (1.7×1.0 m table top).

Drug-induced circling. Saline injection did not alter turning patterns in any experimental or control group (Fig. 15). Rats in group MR continued to circle contralaterally after vehicle administration to a significantly greater extent than ipsilaterally (Fig. 15): the number of turns was approximately equal to that observed under non-injection conditions (Fig. 14, compare with Fig. 15).

Apomorphine potentiated contralateral circling in the MR, PC-VMT, and 6-OHDA groups and elicited turning in the PC-ML and PC-PR animals (Fig. 15). Rotational preferences were not altered by 1 mg/kg apomorphine in the PC group (Fig. 15).

Amphetamine greatly augmented contralateral turning in MR rats at both doses used (Fig. 15). In contrast to the effects of apomorphine, amphetamine produced ipsilateral

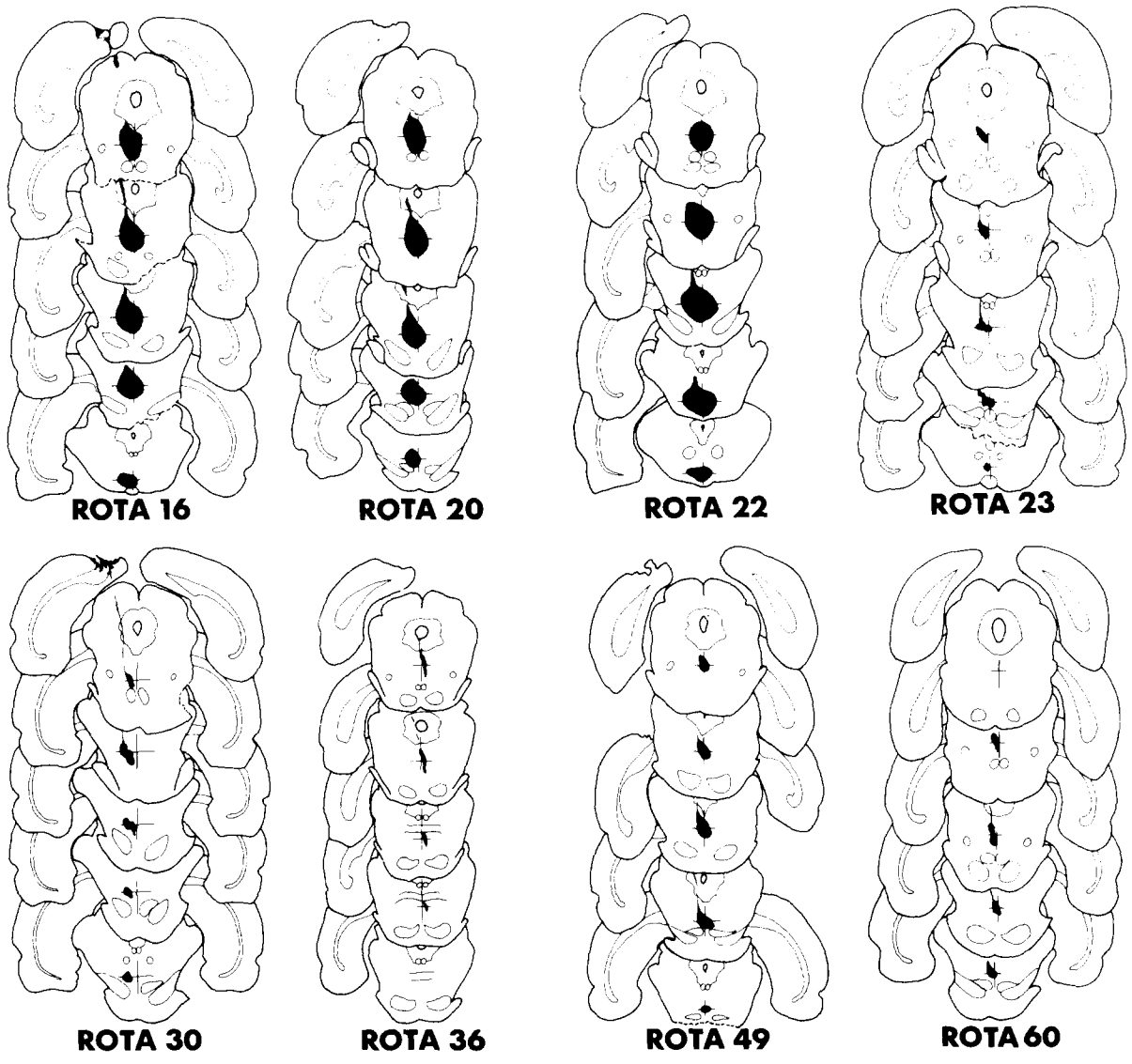

FIG. 11. Radio-frequency lesions representing the range of neuropathologic involvement in group MR. Two rats' histologies are not shown. ROTA 16, 20, and 22 were sacrificed 3 weeks after lesion placement; ROTA 23, 30, 36, 49, and 60 at 24 weeks. For further explanation see text and legends of Fig. 5 and 6.

circling in 6-OHDA-lesion rats (Fig. 15). Although the number of rotations was increased after both doses of amphetamine in the PC group of animals, only at 5 mg/kg was significant ipsilateral turning observed (Fig. 15). A similar behavioral profile was seen in the remaining experimental groups, with the exception of the PC-ML animals, which showed ipsilateral circling after 1.5 mg/kg amphetamine but failed to display a significant turning preference after 5 mg/kg (Fig. 15).

The rotational behavior induced or intensified by drugs was qualitatively different among the different experimental groups. The disparities were most pronounced between radio-frequency-lesion and 6-OHDA-lesion animals. Rats with 6-OHDA lesions pivoted in a constricted manner, often wrapping themselves around the central column of the testing chamber. Animals with radio-frequency lesions displayed circling as traditionally defined (e.g., Fig. 2).

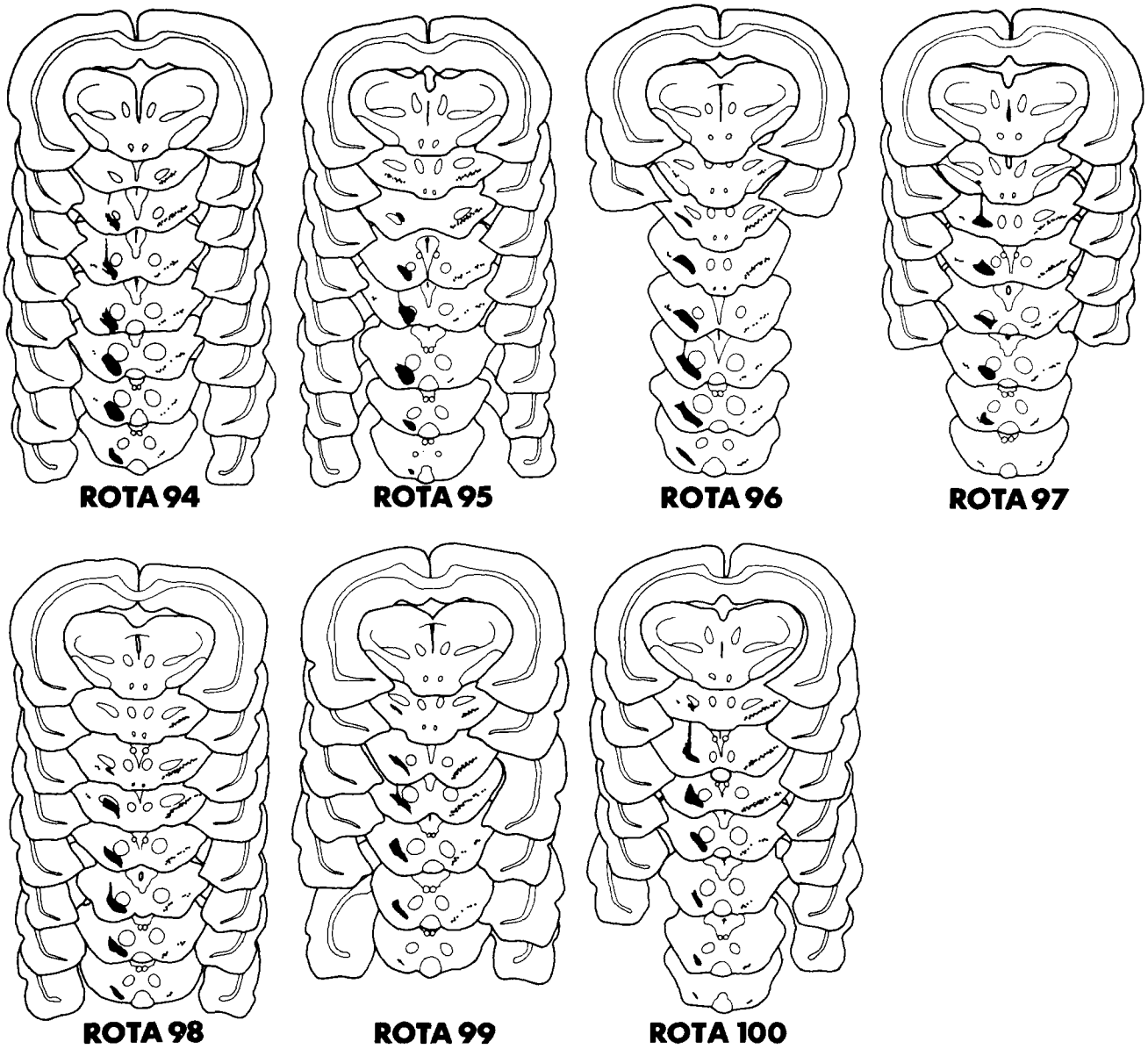

FIG. 12. 6-hydroxydopamine lesions representing the range of neuropathologic involvement in group 6-OHDA. All rats were sacrificed 2 weeks after lesion placement. For further explanation see text and legends of Fig. 4 and 6.

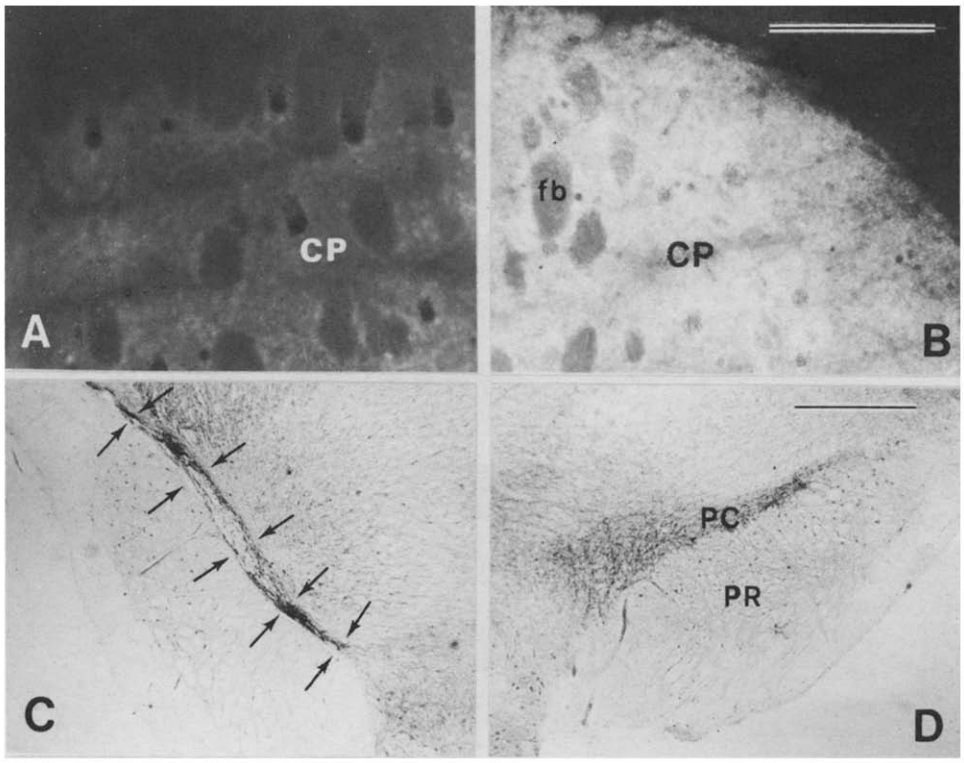

FIG. 13. Extensive loss of dopamine from the caudate-putamen complex (CP, Frame A; compare with Frame B) 2 weeks after a radio-frequency lesion in pars compacta (PC) of the substantia nigra (Frame C). Arrows in C bracket the extent of the ablation. Non-lesioned substantia nigra is shown in D; corresponding fluorescence in CP is displayed in B. Acetylcholinesterase stain in C and D. fb, fiber bundle perforating CP; PR, pars reticulata, substantia nigra. Scale in B is 200 μm and applies also to A; scale in D is 600 μm and applies also to C.

DISCUSSION

Radio-frequency ablations confined almost exclusively to pars compacta of the substantia nigra produced circling contralateral to the lesion side. This turning developed within 8 hr post-surgically and continued for 5 days before returning to control levels. Although in agreement with certain previous reports (e.g., [39]), this finding is at variance with others in which either no spontaneous turning (e.g., [47]) or ipsilateral turning (e.g., [38,44] has been observed in pharmacologically unmanipulated rats after ablations reported to involve dopaminergic neurons in the substantia nigra selectively. The nature of this disparity is open to speculation. Since selective pars compacta lesions reduce caudate-putamen dopamine only partially [22], one possibility is that the remaining dopaminergic innervation, apparently deriving from non-nigral dopamine somata groups in the midbrain (i.e., A8 and A10 configurations; see [10,21]) participated in the production of contralateral circling. The mechanism for this lesion-induced turning is not clear. According to the most current formulations of the involvement of the nigrostriatal system in rotation, the caudate-putamen nucleus on the lesioned side would have to contain the more efficacious dopamine system [24]. Perhaps the partially denervated striatal target cells became supersensitive to the actions of dopamine in the remaining nerve terminals (see [45,47]). The onset of contralateral circling that we observed, however, appears too short for supersensitivity to have developed [45,47]. It appears more likely that increased, injury-induced release of dopamine from the damaged dopamine neurons may have accounted for our observations at shorter postsurgical intervals (see [44]), since complete loss of neostriatal dopamine does not occur until approximately 48 hr after ablations in the substantia nigra and ventromedial mesencephalic tegmentum [44]. Nonetheless, injury-induced release cannot account for all the circling in PC animals, particularly at later times after surgery. We observed circling that persisted for 5 days, approximately 3 days longer than injury-induced release mechanisms would be predicted to

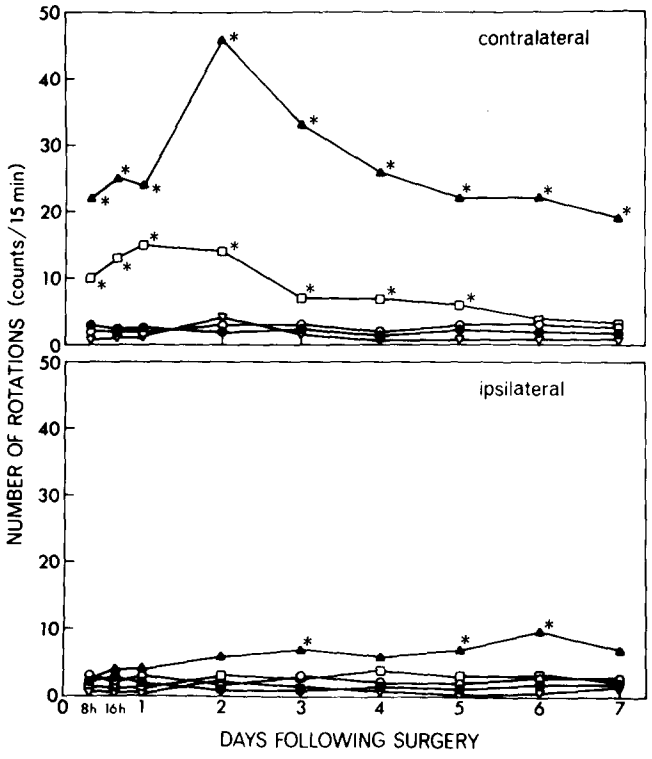

FIG. 14. Spontaneous circling induced by unilateral brainstem lesions. The ranges of standard errors of the means were: non-lesioned controls (○——○), 0.2-0.7; sham-lesioned controls (●——●), 0.2-0.8; group PC (□——□), 0.4-3.7; group MR (▲——▲), 0.9-16.0; group 6-OHDA (▽——▽), 0.3-1.6. *$p<0.05$, compared to sham-lesioned controls. No other experimental group—including, but not shown in the Fig., groups PC-VMT, PC-ML, and PC-PR—displayed circling significantly different from sham-lesioned controls, which in turn did not differ from non-lesioned controls.

operate [44]. The mechanism underlying this later circling is not known.

More important for circling than the nigrostriatal dopamine projection, however, may be neuronal systems penetrating and/or encompassing the median raphe and adjacent reticular formation since rotation was considerably more pronounced and lasted for a longer time after ablations in that region. In fact, contralateral circling in the MR group did not show total recovery and persisted for the duration of the behavioral experiments reported here, at least 3 weeks. It is unlikely that ascending 5-hydroxytryptamine systems to the telencephalon contributed profoundly to turning after lesions in the MR group since ablations encompassing the ventromedial mesencephalic tegmentum (e.g., group PC-VMT), through which course many 5-hydroxytryptamine fibers projecting to forebrain regions [4], did not affect circling. Lesions in the group MR rats might have disrupted inhibitory influences of raphe cells on nigral neurons, however, thereby producing increased dopamine release in the striatum and increased contralateral turning (see [23]). Again, such speculation, based primarily on pharmacologic data, is not strongly supported by recent neuroanatomic observations. Although some reports [19] have suggested a possible median raphe projection to the substantia nigra, other data [5,6] indicate the existence of only a minor, if any, median raphe-nigral projection. Indeed, Pasquier *et al.* [35] have found no histologic evidence of a direct median raphe-substantia nigra projection in the rat (also Pasquier, personal communication).

The drug effects in the present study appear difficult to interpret in terms of current models of circling behavior. Rats in the MR group turned contralaterally both spontaneously and, consistent with the findings of Giambalva *et al.* [23], after apomorphine and amphetamine, although more rotations were observed under drug than control conditions. Perhaps interactions among catecholamine and 5-hydroxytryptamine systems projecting to the caudate-putamen nu-

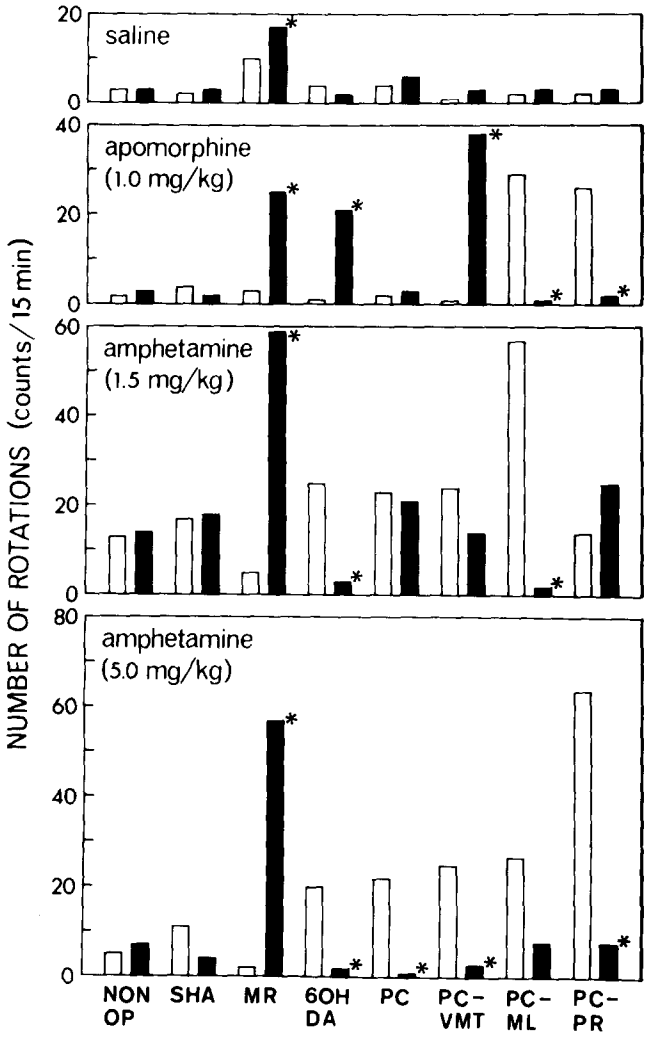

FIG. 15. Effects of drugs on circling. Open bars represent turning ipsilateral to the lesioned side or to the right side of non-lesioned rats; black bars show turning contralateral to the ablated side or to the left in non-lesioned animals. The ranges of standard errors of the mean were: saline, 0.3–5.1; apomorphine, 0.3–20.0; 1.5 mg/kg amphetamine, 0.5–20.0; 5.0 mg/kg amphetamine, 0.4–49.0. *$p<0.05$, compared to turning to other side in same experimental group. NONOP, nonlesioned group; SHA, sham-lesioned group. For other abbreviations see text.

cleus and other telencephalic centers are important for this potentiation (compare [13,14] and [23]), a possibility made more plausible by the observations of Grabowska *et al.* [26] that the locomotor stimulation produced by apomorphine could be potentiated by *p*-chlorophenylalanine and antagonized by 5-hydroxytryptophan.

Animals with 6-OHDA lesions or with combined lesions of the pars compacta and ventromedial mesencephalic tegmentum (group PC-VMT) showed similar turning patterns after apomorphine and amphetamine, with apomorphine eliciting contralateral and amphetamine producing ipsilateral circling. This might be expected since the extent and location of tissue destruction was comparable in the PC-VMT and 6-OHDA groups. The findings with these two groups are also consistent with previous reports (e.g., [45,47]) on similarly placed lesions and can be explained in terms of the "dopamine imbalance" model of rotational behavior (see [24]). If neostriatal dopamine receptors became supersensitive after midbrain lesions [45,47], then contralateral turning would be expected after apomorphine, which we observed, and ipsilateral circling would be predicted after amphetamine, which we also found. Presumably, both pars compacta and the ventral tegmentum must be damaged to produce the "classical" turning syndrome attributed by previous investigators to destruction of the nigrostriatal dopamine system (e.g., [47]).

Since both apomorphine and amphetamine produced ipsilateral turning in the PC-ML and PC-PR groups, the concept of receptor supersensitivity cannot account for the observed drug effects in these animals. In fact, it is possible that an insufficient number of pars compacta somata were destroyed to allow development of receptor supersensitivity in the ipsilateral caudate-putamen nucleus. Indeed, significant portions of pars compacta, particularly at rostral levels, remained after lesions in the PC-ML and PC-PR animals. But why did apomorphine and amphetamine elicit turning in the same direction? If we restrict ourselves to current accepted mechanisms of action of these compounds (e.g., see [24,44]: for caveats see [26]), then one possibility is that ipsilateral neostriatal dopamine receptors became hyposensitive as a function of subtotal lesions in pars compacta.

Ipsilateral rotations were elicited in group PC animals only at the highest dose of amphetamine; apomorphine and 1.5 mg/kg amphetamine did not elicit circling. Although these results can possibly be explained by dose-response considerations and the fact that our pars compacta ablations produced incomplete loss of neostriatal dopamine, the general problem of apomorphine's effects on circling deserve further comment. Despite the fact that many studies in which 6-OHDA was utilized to lesion components of the nigrostriatal system have found marked contralateral turning induced by apomorphine [12, 30, 45, 47], there does not appear to be a consistent response to this drug following nigral lesions. Several authors have noted that, depending on the method of lesioning, either ipsilateral or contralateral turning can be elicited [12, 15, 39, 46]. It is frequently assumed that differences in behavior of this type are attributable to differences in selectivity of destruction of pars compacta afforded by 6-OHDA with respect to other lesion procedures. But contralateral turning to apomorphine has also been found after electrolytic lesions [11,19].

Perhaps one explanation for these diverse findings is that the substantia nigra is not a functionally homogeneous structure: as different regions of the nucleus are ablated (as occurs from study to study) different behavioral sequelae are observed. Indeed, Nashold *et al.* [34] have reported that the direction of circling following carbachol injection into the substantia nigra is dependent upon the site of administration within that structure. Furthermore, different areas of pars compacta of the substantia nigra project to different regions of not only the caudate-putamen complex [10,32] but apparently other forebrain regions as well [21]. This topographic pattern could provide the neuroanatomic basis for different functions of different dopaminergic and other neurochemically identifiable somata in pars compacta and other neural regions.

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REFERENCES

1. Agid, Y., F. Javoy, J. Glowinski, D. Bouvet and C. Sotelo. Injection of 6-hydroxydopamine into the substantia nigra of the rat. II. Diffusion and specificity. *Brain Res.* **58:** 291–325, 1973.
2. Andén, N.-E, A. Dahlström, K. Fuxe and K. Larsson. Functional role of the nigro-neostriatal dopamine neurons. *Acta pharmacol. tox.* **24:** 263–274, 1966.
3. Andén, N.-E., A. Dahström, K. Fuxe, K. Larsson, L. Olson and U. Ungerstedt. Ascending monoamine neurons to the telencephalon and diencephalon. *Acta physiol. scand.* **67:** 313–326, 1966.
4. Bobillier, P., F. Petitjean, D. Salvert, M. Ligier and S. Seguin. Differential projections of the nucleus raphe dorsalis and nucleus raphe centralis as revealed by autoradiography. *Brain Res.* **85:** 205–210, 1975.
5. Bobillier, P., S. Seguin, F. Petitjean, D. Salvert, M. Touret and M. Jouvet. The raphe nuclei of the cat brainstem: A topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res.* **113:** 449–486, 1976.
6. Bunney, B. S. and G. K. Aghajanian. The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. *Brain Res.* **117:** 423–425, 1976.
7. Butcher, L. L. Degenerative processes after punctate intracerebral administration of 6-hydroxydopamine. *J. neural Trans.* **37:** 189–208, 1975.
8. Butcher, L. L. and L. Bilezikjian. Acetylcholinesterase-containing neurons in the neostriatum and substantia nigra revealed after punctate intracerebral injection of di-isopropylfluorophosphate. *Eur. J. Pharmac.* **34:** 115–125, 1975.
9. Butcher, L. L., S. M. Eastgate and G. K. Hodge. Evidence that punctate intracerebral administration of 6-hydroxydopamine fails to produce selective neuronal degeneration: Comparison with copper sulfate and factors governing the deportment of fluids injected into brain. *Naunyn-Schmiedeberg's Arch. Pharmac.* **285:** 31–70, 1974.
10. Butcher, L. L. and K. Talbot. Acetylcholinesterase in rat nigro-neostriatal neurons: Experimental verification and evidence for cholinergic-dopaminergic interactions in the substantia nigra and caudate-putamen complex. In: *Cholinergic-Monoaminergic Interactions in the Brain*, edited by L. L. Butcher. New York: Academic Press, 1978, pp. 25–95.

11. Corrodi, H., K. Fuxe and P. Lidbrink. Interaction between cholinergic and catecholaminergic neurones in rat brain. *Brain Res.* **43:** 397–416, 1972.
12. Costall, B., C. D. Marsden, R. J. Naylor and C. J. Pycock. Differences in circling responses following electrolytic and 6-hydroxydopamine lesions of the nigro-striatal pathway. *Br. J. Pharmac.* **55:** 289P–290P, 1975.
13. Costall, B. and R. J. Naylor. Stereotyped and circling behaviour induced by dopaminergic agonists after lesions of the midbrain raphe nuclei. *Eur. J. Pharmac.* **29:** 206–222, 1975.
14. Costall, B. and R. J. Naylor. A comparison of circling models for the detection of antiparkinson activity. *Psychopharmacologia* **41:** 57–64, 1975.
15. Costall, B. and R. J. Naylor. Actions of dopaminergic agonists on motor functions. In: *Advances in Neurology*, Vol. 9, edited by D. B. Calne, T. N. Chase and A. Barbeau. New York: Raven Press, 1975, pp. 285–297.
16. Costall, B., R. J. Naylor and J. E. Olley. Catalepsy and circling behaviour after intracerebral injections of neuroleptic, cholinergic and anticholinergic agents into the caudate-putamen, globus pallidus and substantia nigra of rat brain. *Neuropharmacology* **11:** 645–663, 1972.
17. Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta. physiol. scand. Suppl. 232* **62:** 1–55, 1965.
18. de la Torre, J. C. and J. M. Surgeon. A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique: The SPG method. *Histochemistry* **49:** 81–93, 1976.
19. Dray, A., L. J. Fowler, N. R. Oakley, M. A. Simmonds and T. Tanner. Comparison of circling behaviour following unilateral inhibition of GABA-transaminase or discrete electrolytic lesioning in the rat substantia nigra. *Br. J. Pharmac.* **55:** 288P, 1975.
20. Dray, A., T. J. Goyne, N. R. Oakley and T. Tanner. Evidence for the existence of a raphe projection to the substantia nigra in rat. *Brain Res.* **113:** 45–57, 1976.
21. Fallon, J. H. and R. Y. Moore. Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection of the basal forebrain and neostriatum. *J. comp. Neurol.* **180:** 545–579, 1978.
22. Faull, R. L. M. and R. Laverty. Changes in dopamine levels in the corpus striatum following lesions in the substantia nigra. *Expl Neurol.* **23:** 332–340, 1969.
23. Giambalvo, C. T., S. R. Snodgrass and N. J. Uretsky. Effects of serotonin neurotoxins on rotational behavior in the rat. *Ann. N.Y. Acad. Sci.* **305:** 524–531, 1978.
24. Glick, S. D., T. P. Jerussi and L. N. Fleisher. Turning in circles: The neuropharmacology of rotation. *Life Sci.* **18:** 889–896, 1976.
25. Glick, S. D., T. P. Jerussi, D. H. Waters and J. P. Green. Amphetamine-induced changes in striatal dopamine and acetylcholine levels and relationship to rotation (circling behavior) in rats. *Biochem. Pharmac.* **23:** 3223–3225, 1974.
26. Grabowska, M., L. Antkiewicz, J. Maj and J. Michaluk. Apomorphine and serotonin neurons. *Pol. J. Pharmac. Pharm.* **25:** 29–39, 1973.
27. Hökfelt, T. and U. Ungerstedt. Specificity of 6-hydroxydopamine induced degeneration of central monoamine neurones: An electron and fluorescence microscopic study with special reference to intracerebral injection on the nigro-striatal dopamine system. *Brain Res.* **60:** 269–297, 1973.
28. Jacquet, Y. F., M. Carol and I. S. Russell. Morphine-induced rotation in naive, nonlesioned rats. *Science* **192:** 261–263, 1976.
29. Karnovsky, M. J. and L. Roots. A "direct-coloring" thiocholine method for cholinesterases. *J. Histochem. Cytochem.* **12:** 219–221, 1964.
30. Kelly, P. H. and R. J. Miller. The interaction of neuroleptic and muscarinic agents with central dopaminergic systems. *Br. J. Pharmac.* **54:** 115–121, 1975.
31. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Wilkins, 1963.
32. Lindvall, O. and A. Björklund. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta physiol. scand. Suppl.* **412:** 1–48, 1974.
33. Muller, P. and P. Seeman. Neuroleptics: Relation between cataleptic and anti-turning actions, and role of the cholinergic system. *J. Pharm. Pharmac.* **26:** 981–984, 1974.
34. Nashold, B. S., Jr., J. R. Urbaniak and M. A. Hatcher. Chemical stimulation of red nucleus, substantia nigra, and basis pedunculi in alert cats. *Neurology* **15:** 604–612, 1965.
35. Pasquier, D. A., T. L. Kemper, W. B. Forbes and P. J. Morgane. Dorsal raphe, substantia nigra and locus coeruleus: Interconnections with each other and the neostriatum. *Brain Res. Bull.* **2:** 323–339, 1977.
36. Poirier, L. J., P. Langelier, A. Roberge, R. Boucher and A. Kitsikis. Non-specific histopathological changes induced by the intracerebral injection of 6-hydroxy-dopamine (6-OH-DA). *J. Neurol. Sci.* **16:** 401–416, 1972.
37. Pycock, C. J., I. MacG. Donaldson and C. D. Marsden. Circling behaviour produced by unilateral lesions in the region of the locus coeruleus in rats. *Brain Res.* **97:** 317–319, 1975.
38. Pycock, C. J. and C. D. Marsden. The rotating rodent: A two component system? *Eur. J. Pharmac.* **47:** 167–175, 1978.
39. Schwartz, W. J., R. H. Gunn, F. R. Sharp and E. V. Evarts. Unilateral electrolytic lesions of the substantia nigra cause contralateral circling in rats. *Brain Res.* **105:** 358–361, 1976.
40. Skinner, J. E. *Neuroscience: A Laboratory Manual.* Philadelphia: Saunders, 1971.
41. Sotelo, C., F. Javoy, Y. Agid and J. Glowinski. Injection of 6-hydroxydopamine in the substantia nigra of the rat. I. Morphological study. *Brain Res.* **58:** 269–290, 1973.
42. Tarsy, D., C. Pycock, B. Meldrum and C. D. Marsden. Rotational behavior induced in rats by intranigral picrotoxin. *Brain Res.* **89:** 160–165, 1975.
43. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand. Suppl.* **367:** 1–49, 1971.
44. Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. *Acta physiol. scand. Suppl.* **367:** 49–68, 1971.
45. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta physiol. scand. Suppl.* **367:** 69–93, 1971.
46. Ungerstedt, U. Discussion. In: *Advances in Neurology*, Vol. 5, *2nd Canadian-American Conference on Parkinson's Disease*, edited by F. H. McDowell and A. Barbeau. New York: Raven Press, 1974, p. 17.
47. Ungerstedt, U. and G. W. Arbuthnott. Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res.* **24:** 485–493, 1970.
48. Ungerstedt, U., L. L. Butcher, S. G. Butcher, N.-E. Andén and K. Fuxe. Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. *Brain Res.* **14:** 461–471, 1969.
49. White, R. P. and H. E. Himwich. Circus movements and excitation of striatal and mesodiencephalic centers in rabbits. *J. Neurophysiol.* **20:** 81–90, 1957.