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Drug-Induced Rotation in Rats without Lesions: Behavioral and Neurochemical Indices of a Normal Asymmetry in Nigro-Striatal Function

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Abstract. Normal unoperated rats were tested for rotation (i.e., circling behavior) in a spherical "rotometer" and dose-response relationships were generated using *d*-amphetamine, apomorphine, L-Dopa, haloperidol, and scopolamine. The rotation induced by amphetamine was significantly antagonized by alpha-methyl-*p*-tyrosine and haloperidol, but not by diethyldithiocarbamate. The rotation elicited by apomorphine was unaffected by alpha-methyl-*p*-tyrosine. Rotation was not necessarily in the same direction with high and low doses of amphetamine, or amphetamine and apomorphine administered a week apart from each other. Dopaminergic-cholinergic interactions were evident, since pilocarpine antagonized amphetamine-induced rotation whereas scopolamine did not; scopolamine elicited rotation in the same direction as that induced by amphetamine. Left and right striatal dopamine and tel-diencephalic norepinephrine levels were determined in rats injected with various doses of amphetamine and tested for rotation. There were significant bilateral differences in striatal dopamine which were related to the direction of rotation. Since amphetamine was found to be equally distributed to the two sides of the brain, the difference in striatal dopamine appeared to be the neurochemical substrate for rotation in normal rats. These results suggest that normal rats have asymmetrical levels of striatal dopamine as well as an asymmetrical complement of striatal dopamine receptors.

Key words: Rotation – *d*-Amphetamine – Apomorphine – Scopolamine – L-Dopa – Haloperidol.

Rats with unilateral lesions of the corpus striatum or the substantia nigra exhibit a postural asymmetry shortly after the operation, which consists of turning ipsilateral to the lesion (Andén et al., 1966; Ungerstedt, 1971 a). This behavior appears to be caused by a functional imbalance of the ascending dopaminergic nigrostriatal pathways ipsilateral and contralateral to the lesion (Andén, 1970; Arbuthnott and Crow, 1971; Costall et al., 1972; Crow, 1971; Ungerstedt, 1971 a; Ungerstedt and Arbuthnott, 1970). Systemic administration of pharmacological agents which mimic the effect of dopamine, either by releasing dopamine (e.g., amphetamine) or by directly stimulating striatal dopamine receptors (e.g., apomorphine), exacerbate the lesion-induced imbalance and cause these rats to rotate or turn in circles. Lesions of the substantia nigra or the corpus striatum destroy presynaptic terminals of ascending nigral efferents; in addition, striatal lesions destroy postsynaptic dopamine receptors. Rats always rotate ipsilateral to a unilateral nigral or striatal lesion after the administration of amphetamine. Following apomorphine administration, however, rats rotate ipsilateral to a striatal lesion but contralateral to a nigral lesion. Rotation always occurs to the side which is functionally least responsive to pharmacological agents (Arbuthnott and Crow, 1971; Ungerstedt, 1971 a; Ungerstedt and Arbuthnott, 1970).

Changes in dopamine content or metabolism have been correlated with rotational behavior following unilateral nigro-striatal lesions. Alpha-methyl-*p*-tyrosine (AMPT), which inhibits tyrosine hydroxylase (Spector et al., 1965; Weissman et al., 1966) and therefore catecholamine synthesis, markedly reduces or completely abolishes amphetamine-induced rotation but does not affect rotation elicited by apomorphine. Haloperidol, which has a high dopaminergic to noradrenergic blocking ratio (Andén et al., 1970), prevents the rotation induced by amphetamine as well as that elicited by apomorphine. On the other hand, rats pretreated with FLA63, an inhibitor of dopamine-beta-hydroxylase (Corrodi et al., 1970) which selec-

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tively blocks norepinephrine synthesis, showed no decrease in rotation after the administration of amphetamine (Andén, 1970; Christie and Crow, 1971; Ungerstedt, 1971a).

Although it appears that monoamines other than dopamine are not primarily involved in the drug-induced rotation in rats with unilateral lesions of the nigro-striatal system (Andén et al., 1966; Marsden and Guldberg, 1973), the cholinergic influences on rotation appear to be appreciable. Rats receiving unilateral intrastriatal injections of atropine (Costall et al., 1972), amphetamine, or apomorphine (McKenzie et al., 1972; Ungerstedt et al., 1969) exhibit contraversive turning. When the cholinergic agonist arecoline (Costall et al., 1972) or the anticholinesterase neostigmine (McKenzie et al., 1972) is administered by the same technique, rotation in the opposite direction is observed. In unilaterally striatotomized rats, turning of the head and tail to the unoperated side after the intraperitoneal injection of haloperidol is antagonized by the systemic administration of scopolamine (Andén and Bedard, 1971), and scopolamine also antagonizes the chlorpromazine block of amphetamine-elicited turning in rats with unilateral substantia nigra lesions (Muller and Seeman, 1974). Thus it appears that the dopaminergic and cholinergic systems are in reciprocal balance. While dopaminergic mechanisms turn the animal in one direction, cholinergic mediation tends to counteract this action by moving the animal in the opposite direction.

Previously we reported that *d*-amphetamine (Jerussi and Glick, 1974) and apomorphine (Jerussi and Glick, 1975) could elicit rotation in normal unoperated rats and suggested that there may exist an intrinsic bilateral imbalance in the functioning of the nigrostriatal pathways of normal rats. The present investigation describes the dose-related rotation induced by dopaminergic and cholinergic agonists and antagonists in normal rats, and the neurochemistry establishing an asymmetric effect of amphetamine on striatal dopamine content correlative to the direction of amphetamine-induced rotation.

BEHAVIORAL METHODS

Female Sprague-Dawley rats about 3 months of age and ranging in weight from approximately 200 to 300 g were used in all the experiments. The animals were individually tested for rotation in one of two identical modified "rotometers" (Glick and Greenstein, 1973) described initially by Ungerstedt and Arbuthnott (1970). The apparatus consisted of a white opaque plexiglas sphere, 30.5 cm in diameter, within which the rat rotated. A flexible stainless steel wire, which was connected to a cam positioned on the vertical axis on the outer surface of the sphere, was wrapped around the thorax of the animal and clipped to itself. As the rat rotated, the cam closed one of the two microswitches which were positioned as to indicate left or right turns. Generally, 15 min after the rat had been placed in the apparatus, it was injected i.p. with the test drug or its diluent. Rotations were automatically recorded on a print-out counter at 5-min intervals, for the 15 min before and 60 min after injection. Rotations to the left or right during the pre-injection and post-injection periods were separately totalled and net positive rotations (i.e. rotations in the dominant direction minus rotations in the opposite direction) were determined for each rat. In order to avoid spurious correlations, only rats which displayed sufficient rotation (i.e. net rotations greater than or equal to 10/h) were selected for repeated testing. In these consistency and interaction expriments, rats under the same or different pharmacological conditions were tested twice for rotation with an interval of 1 week separating the two testing sessions (Day 1 and Day 8).

The following drugs were used: dextroamphetamine sulphate; apomorphine hydrochloride; dihydroxyphenylalanine (L-Dopa); scopolamine hydrobromide; pilocarpine nitrate; DL-alpha-methyl-*p*-tyrosine methyl ester hydrochloride (AMPT); diethyldithiocarbamate (DDC); beta-(3,4-dihydroxyphenyl)-alpha-hydrazino-alpha-methyl propionic acid (MK 486—Merck, Sharp & Dome); haloperidol. Haloperidol was injected as a fine suspension. Distilled water was used as the diluent for apomorphine (injection volume = 2.0 ml/kg). MK 486 and L-Dopa (injection volume = 4.0 ml/kg) were dissolved in physiological saline (0.9%) adjusted to pH 1. All other pharmacological agents were dissolved in physiological saline and injected in a volume of 1.0 ml/kg. Rats were pretreated for 30, 45, 60, or 135 min when MK 486 (150.0 mg/kg), DDC (200.0 mg/kg), haloperidol (1.0 mg/kg), or AMPT (150.0 mg/kg), respectively, were used prior to the administration of a second drug.

NEUROCHEMICAL METHODS

Sixty-three rats were individually placed in a rotometer, and after 15 min were injected i.p. with one of the following doses of amphetamine: 0.0 (saline), 2.0, 5.0, 10.0, 20.0 mg/kg. Seven to fourteen rats were tested at each dose of amphetamine, whereas 22 animals comprised the saline control group. Rotations were recorded for 30 min after the administration of either amphetamine or saline, and net rotations were determined for each rat. One minute after being taken out of the apparatus, each rat was killed by decapitation, and the excised brain was dissected into 4 parts: left and right striatum, left and right remaining tel-diencephalon. The cerebellum and brainstem were discarded.

The four parts of the brain were individually homogenized in 5 and 10 ml of 0.4 N $HClO_4$ (plus 10 mg of EDTA) for the striata and the tel-diencephalon halves, respectively. After centrifuging the samples at 27000 × g for 20 min, additional (10 mg) EDTA was added to the supernatant which was subsequently adjusted to pH 8.4 and then passed through a column of alumina according to the procedure of Weil-Malherbe (1971). The fraction collected from the 0.2 M acetic acid eluent was adjusted to pH 6.5 with 1.0 M dibasic potassium phosphate solution (Snyder and Taylor, 1972) and then striatal dopamine and tel-diencephalic norepinephrine were determined by the spectrofluorometric method (Laverty and Taylor, 1968). The initial eluate of the column, however, was collected, frozen, and later assayed for amphetamine according to the gas chromatographic method of Waters (in preparation) as modified from Noonan et al. (1969) and Anggard et al. (1970).

For the determination of amphetamine, initially all samples were acidified (pH 2) with 2.0 ml 0.1 N H_2SO_4. The concentration (in μg/ml) of the internal standard, benzylamine which was added next to each striatum or tel-diencephalon sample, was determined by multiplying either 10^{-4} or 10^{-3}, respectively, by the dose of amphetamine administered to the rats. External standards of amphetamine were prepared in duplicate at three different concentrations: one-half, equal to, and two times, the concentration of benzylamine. The amphetamine standards were run through the

entire extraction procedure and treated the same as the unknowns. After the addition of 10 ml of benzene and 1.5 g NaCl, the samples were shaken for 10 min. The tubes were then centrifuged at about 500 × g for 5 min, and the organic phase was removed by aspiration. Now samples were made alkaline (pH 12) by the addition of 2.0 ml 5.0 N NaOH. After adding additional NaCl and another 10 ml of benzene, shaking and centrifuging the samples, all of the benzene phase was transferred to another tube, to which 0.05 ml of trichloroacetylchloride was added. Ten minutes later, 10 ml of 1.0 N NaOH was added and the tubes were shaken for 5 min. The benzene phase was then transferred to another tube and evaporated. The residue was reconstituted usually in about 1.0 ml of benzene and injected (1–5 µl) into a model 7620A Hewlett Packard research chromatograph. Detection by electron capture (^{63}Ni) in the pulsed mode (50 µs pulse interval) was performed under the following conditions: column – 4 feet glass, 4 mm I.D., 6 mm O.D., 3% UCW-98 on Chromsorb W AW-DMCS high performance, 80/100 mesh; temperatures – 250° C (inlet), 175° C (oven), 275° C (detector); carrier gas-5% argon-10% methane, 55 ml/min (flow).

BEHAVIORAL RESULTS

Amphetamine Dose-Response. Figure 1 is the dose-response curve generated after the administration of amphetamine. At all doses, except 0.625 and 5.0 mg/kg, rotations are significantly greater ($P < 0.01$ to $P < 0.001$, *t*-tests) for amphetamine-treated rats as compared to the saline-injected controls. There appears to be a biphasic dose-response relationship with peak rotations occurring at 1.25 and 20.0 mg/kg. The 1.25 mg/kg dose had its peak effect between 25 and 45 min, whereas the 20.0 mg/kg dose induced the greatest rotation within the first 5 min after injection. The 20.0 mg/kg dose produced more variability from animal to animal and more frequent rotations opposite to the initial direction.

Amphetamine Dose-Response Interactions. Two-way analyses of variance, comparing net rotations induced by amphetamine alone to those elicited by the various drug pretreatments (Table 1), indicated that although there were no significant ($P > 0.1$) pretreatment effects, the effects of dose were significant ($P < 0.01$). However, significant dose × pretreatment interactions for AMPT ($P < 0.05$) and haloperidol ($P < 0.01$) necessitated further analysis using multiple *t*-tests (i.e., amphetamine alone vs. amphetamine + AMPT or haloperidol). On the other hand, the lack of a significant ($P > 0.1$) dose × treatment interacton for DDC pretreatment, indicated that DDC had no significant effect on the rotation induced by amphetamine. In animals pretreated with either AMPT or haloperidol, mean net positive rotations were significantly ($P < 0.05$) reduced at doses of 1.0 and 2.5 mg/kg of amphetamine; at higher doses of amphetamine (i.e., greater than 5.0 mg/kg) rotations were not lowered significantly ($P > 0.1$). The time course of rotation induced by high doses of amphetamine (i.e., 25.0 mg/

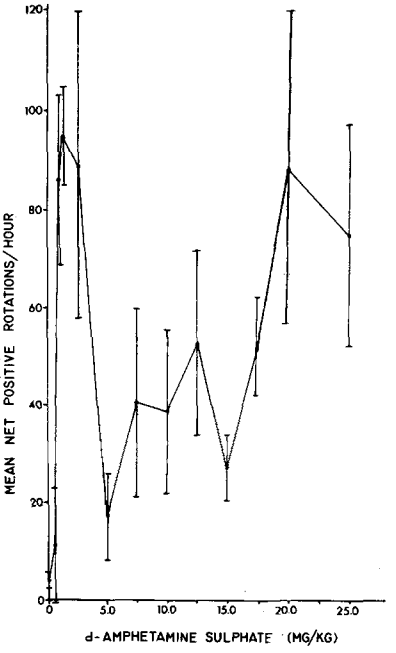

Fig. 1. Dose-response relationship for *d*-amphetamine-induced rotation (mean ± S.E.; $N = 6$ per dose)

Table 1. Amphetamine dose-response interactions

Amphetamine dose	No treatment	(150 mg/kg)-AMPT	(1 mg/kg)-Haloperidol	(200 mg/kg)-DDC
0.0	3.8 ± 1.7	1.8 ± 0.9	0.8 ± 0.5	13.0 ± 4.5
1.0	85.0 ± 19.9	10.7 ± 3.5[a]	0.8 ± 0.3[a]	156.0 ± 68.4
2.5	88.7 ± 31.9	12.0 ± 4.9[b]	0.8 ± 0.3[b]	61.7 ± 24.1
5.0	17.2 ± 9.0	20.3 ± 5.9	3.7 ± 1.1	33.3 ± 11.8
15.0	27.2 ± 7.1	52.3 ± 16.0	34.2 ± 11.3	121.0 ± 26.3
25.0	74.7 ± 22.5	51.7 ± 18.8	57.2 ± 11.4	87.0 ± 21.6

Mean net positive rotation ± S.E. for groups of 6 rats with different amphetamine doses (rows) and different other treatments (columns) tested for their interactions with amphetamine.

[a] $P < 0.005$ for difference from no other treatment (*t*-test).

[b] $P < 0.05$ for difference from no other treatment (*t*-test).

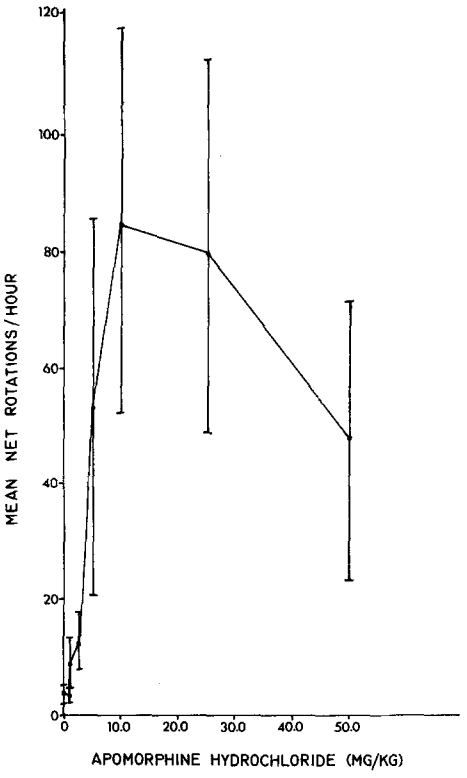

Fig. 2. Dose-response relationship for apomorphine-induced rotation (mean ± S.E.; $N = 6$ per dose)

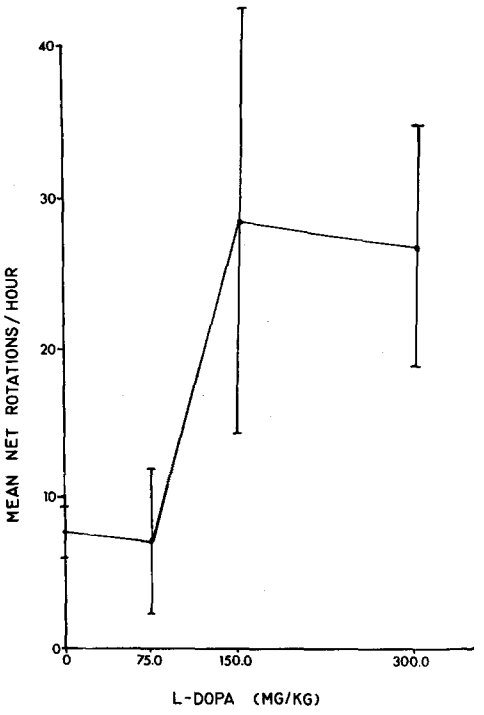

Fig. 3. Dose-response relationship for L-Dopa-induced rotation (mean ± S.E.; $N = 6$ per dose). Fifteen minutes after the administration of MK 486 (150.0 mg/kg), rats were placed in the rotometer 15 min prior to the injection of L-Dopa

kg) alone was markedly altered by the prior administration of AMPT or haloperidol; the rapid onset of rotation following the administration of amphetamine was prevented and the period of peak rotation was shifted to the earlier portion of the test session. Rotations opposite to the initial direction, characteristic of the high doses of amphetamine, were reduced by AMPT or haloperidol pretreatment.

Apomorphine Dose-Response. In Figure 2, the dose-response relationship for apomorphine-induced rotation, all doses equal to or greater than 2.5 mg/kg elicited significant ($P < 0.05$, t-test) rotation. The curve appears to plateau at 10.0 mg/kg since there is no significant ($P > 0.1$, t-test) difference between the effects of 10.0 and 50.0 mg/kg. The rotation induced by 10.0 mg/kg of apomorphine reached its maximum during the first 5 min after injection, and the direction of rotation remained constant throughout the entire hour.

L-Dopa Dose-Response. In Figure 3, only the dose of 300.0 mg/kg of L-Dopa induced significant ($P < 0.05$, t-test) rotation in rats pretreated with MK 486. The inhibitor alone did not cause any significant ($P > 0.1$, t-test) rotation as compared to the saline controls of Figure 1. The time course of the rotation induced by L-Dopa (300.0 mg/kg) was marked by periods of frequent rotations to the opposite direction, and during any 5-min period mean rotations never exceeded five.

Haloperidol Dose-Response. Haloperidol-induced rotation, illustrated in Figure 4, is characterized by a steep dose-response relationship. Only the dose of 0.125 mg/kg elicited significant ($P < 0.05$, t-test) rotation. Rotations, at all other doses, are not significantly ($P > 0.1$, t-test) different from those of the control group. The time course of haloperidol-induced rotation indicated that although the direction of rotation was reasonably constant, the magnitude of rotation was rather minimal. Very few rotations were elicited during any 5-min period.

Scopolamine-Dose-Response. At all doses of scopolamine in Figure 5, the rotation induced was significantly greater than that of the saline controls. The curve appears to plateau at 1.0 mg/kg since there is no significant ($P > 0.1$ for 1.0 vs. 200.0 mg/kg, t-test) difference in effect over a dose range of greater than two log units. The time course of scopolamine-induced rotation showed that the drug did not elicit marked rotation during any of the 5-min periods.

Pilocarpine Dose-Response. Pilocarpine (1.0, 10.0, 100.0, 200.0 mg/kg; $N = 2$ per dose) did not induce any significant rotational behavior. At all doses

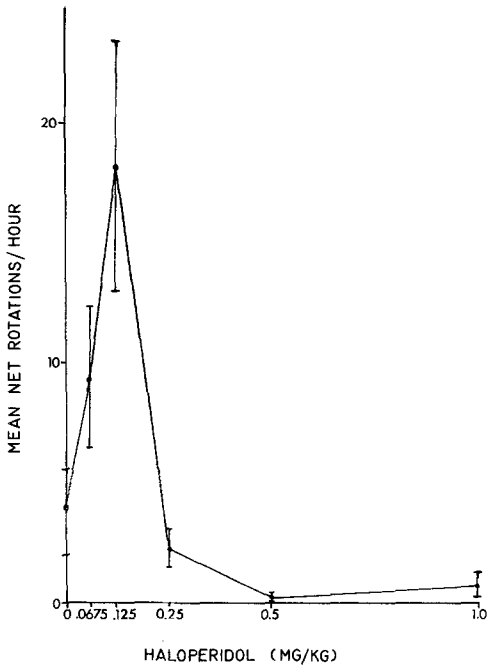

Fig. 4. Dose-response relationship for haloperidol-induced rotation (mean ± S.E.; $N = 6$ per dose). Forty-five minutes after the administration of haloperidol, rats were placed in the rotometer. Fifteen minutes later, rotations were recorded for 60 min

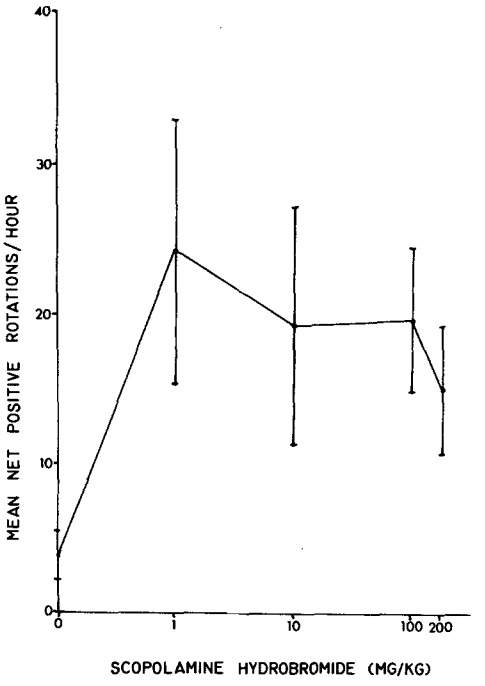

Fig. 5. Dose-response relatonship for scopolamine-induced rotation (mean ± S.E.; $N = 6$ per dose)

tested, mean net rotations were less than 5 during the test hour.

Consistency of and Interactions between Drugs. The direction of rotation induced by amphetamine (1.0 mg/kg), apomorphine (10.0 mg/kg), or scopolamine (1.0 mg/kg) was consistent from week to week (Table 2). Some rats predominantly rotated to the left, whereas others consistently rotated to the right. Only one of the rats treated with scopolamine on Day 8 reversed its initial (Day 1) direction. Of the total rotations on Days 1 and 8, mean percent rotations (± S.D.) in the dominant direction were 91.1% ± 10.1, 88.2% ± 12.0, and 78.0% ± 15.0 for animals tested with amphetamine, apomorphine, and scopolamine, respectively. Not only was the direction of rotation consistent, but the magnitude of rotation induced by the weekly administration of either amphetamine or apomorphine was significantly correlated and not significantly different from Day 1 to Day 8. In contrast the magnitude of rotation elicited by the weekly administration of scopolamine was not significantly correlated and was significantly greater on Day 8 than on Day 1.

The rotation induced by the 1.0 and 20.0 mg/kg doses of amphetamine was not necessarily in the same direction for both doses (Table 2). During the first few minutes of the test session, however, rats tested with 20.0 mg/kg rotated in the same direction as with 1.0 mg/kg, but during the later portions of the 20.0 mg/kg session, a preponderance of rotations to the opposite direction was recorded.

As shown in Table 2, apomorphine (10.0 mg/kg) administered on Day 1 and amphetamine (1.0 mg/kg) on Day 8 did not necessarily induce rotation in the same direction. On the other hand, rats tested with scopolamine (1.0 mg/kg) on Day 1 and amphetamine (1.0 mg/kg) on Day 8 did rotate in the same direction.

Although the direction of rotation induced by 1.0 mg/kg of amphetamine on Day 1 remained unchanged after pretreating rats with AMPT (Day 8), the magnitude of rotation was significantly reduced (Table 2). In contrast, AMPT pretreatment had no significant effect on the direction and magnitude of rotation induced by apomorphine.

The rotation induced by the combination of amphetamine (1.0 mg/kg) and scopolamine (1.0 mg/kg) on Day 8 remained unchanged in direction and was 25% greater (though not significant) than the rotation elicited by amphetamine alone on Day 1 (Table 2). On the other hand, as shown in Table 2, pilocarpine (1.0 mg/kg) significantly reduced the magnitude of rotation induced by amphetamine (1.0 mg/kg) without affecting its direction.

Table 2. Consistency of and interactions between drugs

N	Mean net positive rotations ± S.D.		Percent rotating in same direction as Day 1	
	Day 1 Drug and dose (mg/kg)	Day 8 Drug and dose (mg/kg)		
	Amphetamine (1.0)	Amphetamine (1.0)		
15	92.0 ± 58.9	93.9 ± 58.1	100.0[e]	0.51[b]
	Amphetamine (1.0)	Amphetamine (20.0)		
9	69.8 ± 57.4	45.3 ± 34.5	55.6	0.07
	Amphetamine (1.0)	Amphetamine (1.0) + AMPT (150.0)		
6	126.3 ± 68.2	26.7 ± 25.2[d]	100.0[g]	0.67
	Amphetamine (1.0)	Amphetamine (1.0) + Scopolamine (1.0)		
8	109.4 ± 57.0	137.4 ± 73.6	100.0[g]	–
	Amphetamine (1.0)	Amphetamine (1.0) + Pilocarpine (1.0)		
8	42.0 ± 16.7	9.8 ± 8.9	100.0[g]	–
	Apomorphine (10.0)	Apomorphine (10.0)		
19	79.4 ± 63.8	93.8 ± 77.3	100.0[e]	0.84[a]
	Apomorphine (10.0)	Amphetamine (1.0)		
14	60.2 ± 46.0	31.4 ± 20.8	50.0	–
	Apomorphine (10.0)	Apomorphine (10.0) + AMPT (150.0)		
6	115.5 ± 69.7	89.5 ± 69.5	100.0[g]	0.86[b]
	Scopolamine (1.0)	Scopolamine (1.0)		
13	38.3 ± 21.2	91.8 ± 35.0[c]	92.3[f]	0.23
	Scopolamine (1.0)	Amphetamine (1.0)		
7	88.0 ± 12.4	113.0 ± 60.6	100.0[g]	–

Linear regression analysis
[a] = $P < 0.001$
[b] = $P < 0.05$

Paired t-tests
[c] = $P < 0.001$
[d] = $P < 0.01$

Chi square
[e] = $P < 0.0005$
[f] = $P < 0.01$
[g] = $P < 0.05$

NEUROCHEMICAL RESULTS

In Figure 6, the amphetamine content in the brain after 30 min is linearly related to the dose of amphetamine sulphate administered. Linear regression analysis showed that this relationship applied to both the striata ($r = 0.97$, $P < 0.001$) and tel-diencephalon ($r = 0.98$, $P < 0.001$) samples. On the other hand, Figure 7 indicates that striatal dopamine and tel-diencephalic norepinephrine levels, as a function of the injected dose of amphetamine, was non-monotonic. Norepinephrine was significantly (t-tests) raised 15% and 9%, respectively, above control levels for the 2.0 ($P < 0.05$) and 5.0 ($P < 0.005$) mg/kg doses of amphetamine. However, 20.0 mg/kg of amphetamine resulted in a significant ($P < 0.001$) 47% depletion of norepinephrine. Similarly, doses of 2.0 and 5.0 mg/kg significantly raised dopamine levels 15% ($P < 0.01$) and 38% ($P < 0.001$), respectively. The dose of 10.0 mg/kg also resulted in a significant ($P < 0.01$) 16% increase in dopamine. As with norepinephrine, significant ($P < 0.001$) depletion (36%) of dopamine resulted after the administration of 20.0 mg/kg of amphetamine.

There were no significant left-right differences for the neurochemical levels at each dose of amphetamine ($P > 0.1$, paired t-tests). Moreover, left-right differences for striatal and tel-diencephalic amphetamine were not significant ($P > 0.1$, paired t-tests). When the drug and neurochemical levels were analyzed with respect to the direction of rotation (Table 3), only dopamine differences contralateral and ipsilateral to the direction of rotation at doses of 2.0 and 20.0 mg/kg were significant ($P < 0.05$ and 0.001 respectively, paired t-tests).

DISCUSSION

As evident from the dose-response relationships, pharmacological agents which mimic the effects of dopamine or block the action of ACh induce significant rotation in normal rats. The large standard errors shown in all dose-response curves indicate that the magnitude of rotation varies greatly among rats, and a small percentage (10–20%) do not display sufficient rotations (i.e., net rotations greater than or equal to 10 per hour) to be distinguished from random movement. In spite of the large variability in rotation

between animals, the direction and magnitude of rotation are extremely stable for each individual rat.

Amphetamine-Induced Rotation. For amphetamine-induced rotation, the behavior elicited by the low

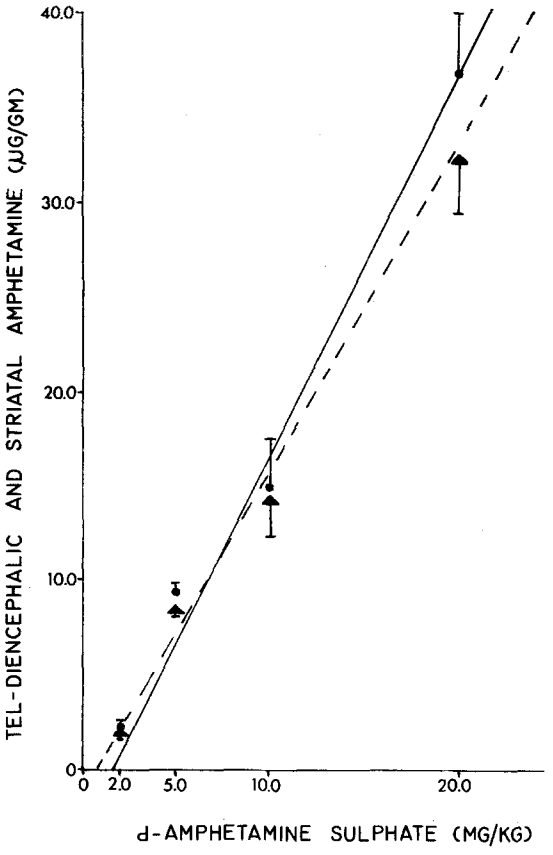

Fig. 6. Mean (+ or −S.D.) tel-diencephalic (●——●) and striatal (▲----▲) amphetamine levels following injection of *d*-amphetamine sulphate

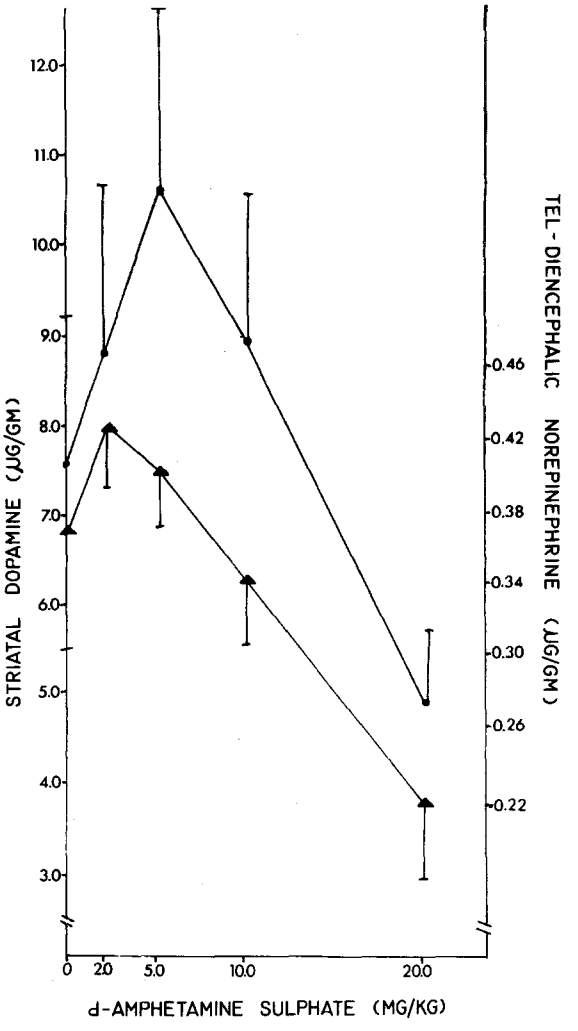

Fig. 7. Mean (+ or −S.D.) tel-diencephalic norepinephrine (▲----▲) and striatal dopamine (●——●) levels following injection of *d*-amphetamine sulphate

Table 3. Mean (± standard deviation) unilateral levels[a] (µg/g) of amphetamine, tel-diencephalic norepinephrine, and striatal dopamine

Amphetamine sulphate (mg/kg)		Amphetamine		Norepinephrine Tel-diencephalon	Dopamine striatum
		Tel-diencephalon	Striatum		
0.0 (saline)	C	–	–	0.38 ± 0.06	7.48 ± 1.58
	I	–	–	0.37 ± 0.07	7.87 ± 1.44
2.0	C	2.11 ± 0.98	2.28 ± 0.96	0.44 ± 0.03	8.32 ± 1.75
	I	2.16 ± 0.84	2.23 ± 0.92	0.42 ± 0.04	9.32 ± 1.98[c]
5.0	C	8.63 ± 3.38	9.79 ± 3.48	0.41 ± 0.03	10.92 ± 2.33
	I	8.42 ± 3.02	8.99 ± 3.24	0.40 ± 0.03	10.21 ± 1.86
10.0	C	15.25 ± 2.57	15.10 ± 2.14	0.35 ± 0.04	9.15 ± 0.92
	I	14.79 ± 2.56	13.53 ± 1.59	0.34 ± 0.03	8.59 ± 1.20
20.0	C	37.39 ± 3.20	32.04 ± 2.74	0.20 ± 0.04	5.40 ± 0.47[b]
	I	36.17 ± 3.06	32.44 ± 3.26	0.20 ± 0.05	4.35 ± 0.67

[a] Mean amphetamine, norepinephrine, and dopamine were computed contralateral (C) and ipsilateral (I) to the direction of rotation.
[b] $P < 0.001$ for difference between C and I (paired *t*-test).
[c] $P < 0.05$ for difference between C and I (paired *t*-test).

doses appeared to be qualitatively different from rotation elicited at the high doses. At low doses the rats reared on their hind limbs and rotated by moving their front paws around the inner surface of the spherical rotometer. However, at high doses of amphetamine, the rats rotated in tight circles very similar to the amphetamine-induced rotation occurring with lower doses in rats with unilateral caudate or substantia nigra lesions. Previous investigators did not report drug-induced rotation in normal rats which were tested on a flat surface (Christie and Crow, 1971; Marsden and Guldberg, 1973; Naylor and Olley, 1972) or in a hemispherical rotometer (Ungerstedt, 1971a; Ungerstedt and Arbuthnott, 1970). Thus it appeared that the spherical nature of the apparatus was an important factor in eliciting rotation (Jerussi and Glick, 1974). Recently, however identical results have been observed (Glick et al., in preparation) on a flat surface when testing was conducted in an enclosed environment (i.e., a 30×30 cm^2 plexiglas box). Rotation cannot be attributed to hyperactivity. That is, if an animal were only hyperactive there might be more rotations, but there is no reason why such rotation should be unevenly distributed, in any consistent way, to the left or right. In fact, net rotations appear to be inversely correlated with locomotor activity (Greenstein and Glick, 1975).

If amphetamine elicits rotation in rats with unilateral lesions of the nigro-striatal system by releasing more striatal dopamine from one side as compared to the other, a similar mechanism could be proposed to account for the biphasic relationship of amphetamine-induced rotation in intact rats (Fig. 1). In normal rats, amphetamine could similarly release more dopamine from one striatum, if one striatum intrinsically contained more dopamine or if its transmitter pool were more susceptible to the releasing action of the drug. Presumably under normal physiological conditions, unequal release from either the left or right striatum would not result in a large enough bilateral postsynaptic imbalance of dopamine to cause any measurable rotation. However, small doses of amphetamine (1.0 mg/kg) could induce rotation by augmenting the normal unequal release and thereby increasing the postsynaptic imbalance of dopamine.

With large doses of amphetamine (20.0 mg/kg) the side that initially released more dopamine would be depleted more quickly and to a greater extent than the contralateral striatum where now the presynaptic pool of dopamine would be relatively large. Thus, the initial dopamine asymmetry would be reversed, i.e. the contralateral striatum would release relatively more dopamine and produce, postsynaptically, a large bilateral imbalance. As a consequence, the animal would reverse its direction of rotation. This proposal is supported by the fact that during the last 30 min of the 20.0 mg/kg test session, a preponderance of rotations opposite to the initial direction was recorded. Moreover, in contrast to the consistent direction of rotation observed after the weekly administration of 1.0 mg/kg of amphetamine, 20.0 mg/kg did not necessarily induce rotation in the same direction as the 1.0 mg/kg dose (Table 2). At intermediate doses of amphetamine (5.0 – 15.0 mg/kg), the initial presynaptic imbalance would be small or only partially reversed. At these doses, net rotations would be minimal (Fig. 1) since the animal would not rotate in any consistent direction.

The AMPT, haloperidol, and DDC dose-response interactions (Table 1) give evidence that the rotation induced in normal rats by amphetamine is mediated by dopamine. DDC, an inhibitor of dopamine beta hydroxylase (Carlsson et al., 1966; Collins, 1965; Goldstein, 1966; Lippmann and Lloyd, 1969), did not inhibit rotation induced by amphetamine. On the other hand, both AMPT and haloperidol significantly reduced the rotation elicited by the low doses (i.e. 1.0 and 2.5 mg/kg) of amphetamine, and the time course of rotation elicited by 25.0 mg/kg of amphetamine in rats pretreated with AMPT or haloperidol was altered. Both AMPT and haloperidol prevented the initial burst of rotation following 25.0 mg/kg of amphetamine. In fact, the time courses of the combined AMPT-amphetamine and haloperidol-amphetamine treatments bared a closer resemblance to the effects of the lower doses of amphetamine.

It is evident from Figure 6 that the tel-diencephalic and striatal dose-response relationships are nearly identical. The amphetamine content in both neuroanatomical structures can be described adequately by the same linear equation: $\mu g/g = 1.76\,(\text{dose}) - 1.16$. Moreover, it appears that in the rat brain, there is no unequal regional distribution of amphetamine between the structures examined, and in addition there are no significant left-right differences for each individual structure.

In the present investigation, doses of *d*-amphetamine from 2.0 to 10.0 mg/kg significantly increased brain catecholamine levels, whereas the dose of 20.0 mg/kg resulted in a significant depletion. High doses of *d*-amphetamine (i.e. greater than 5.0 mg/kg) reduce brain catecholamines (Brodie et al., 1970; Leonard and Shallice, 1971; Glick et al., 1974) presumably by the releasing and consequent depleting action of the drug. However, the mechanism whereby small doses of *d*-amphetamine increase cerebral catecholamines (Smith, 1965; Leonard and Shallica, 1971; Harris et al., 1975) is unclear at present but may involve stimulation of dopamine biosynthesis (Kuczenski, 1975).

Doses of *d*-amphetamine at or near the peaks of rotation in Figure 1 induced significant bilateral differences in striatal dopamine content, contralateral and ipsilateral to the direction of rotation. Bilateral differences between other neurochemical measures were not significant. These observations support the hypothesis that amphetamine-elicited rotational behavior in normal rats is due to a bilateral imbalance of striatal dopamine.

Although it is not clear, from the present investigation, whether amphetamine induced the imbalance or exacerbated a neurochemical asymmetry normally present in rats, Zimmerberg et al. (1974) showed that dopamine levels were significantly higher in the striatum contralateral to the rat's direction of preference in a T-maze, and rotation was in the same direction as this spatial preference. The present findings demonstrate that normal rats, as lesioned rats, rotate to the side containing less dopamine following a 20.0 mg/kg dose of amphetamine. However, following 2.0 mg/kg, normal rats rotate toward the side containing more dopamine. At the latter dose, the amphetamine-induced increase of dopamine in nigro-striatal terminals may be partially counteracted by the drug's releasing action. However, due to the unequal release of dopamine from the two striata, as previously suggested, the side more susceptible to amphetamine's releasing action would contain less presynaptic dopamine but more of the amine postsynaptically. Thus the animal would rotate to the side where postsynaptic dopamine was less. Since, postsynaptically, the amine would be more susceptible to catabolism (Axelrod, 1970) and transport out of the striatum, neurochemical analysis at this time would indicate higher striatal dopamine ipsilateral and not contralateral to the direction of rotation. Initially, with high doses of amphetamine, dopamine would be rapidly depleted from the striatum which was more susceptible to amphetamine's releasing action. As the other striatum began to release proportionally more of the amine, the animal would reverse its direction of rotation. At this time, neurochemical determinations would indicate a higher level of dopamine in the striatum contralateral to the direction of rotation.

Apomorphine-Induced Rotation. Amphetamine-induced rotation in normal rats was explained by a mechanism which involved the asymmetrical or unequal release of dopamine from the left and right striata. Along with this explanation there is the assumption that an intrinsic bilateral neurochemical imbalance of the content or metabolism of dopamine exists in the nigro-striatal pathways of rats. Still it is the unequal dopamine content at the receptor which effects rotation. Because of apomorphine's predominantly postsynaptic mechanism of action (Andén, 1970; Bunney et al., 1973; Ernst, 1965, 1967; Ungerstedt, 1971a), the rotation elicited by the dopaminergic agonist points to the existence of an intrinsic postsynaptic asymmetry in the nigro-striatal systems of normal rats. Either there are more receptors in one striatum as compared to the other, or receptors in one striatum are more sensitive than those in the other. With small doses of apomorphine, differential stimulation of the receptors in the two striata would occur and reach a maximum as the dose was increased to 10.0 mg/kg, at which dose all receptors would appear to be saturated. At this point, the magnitude of rotation would plateau (Fig. 2). Similar to the rotation elicited by amphetamine, apomorphine-induced rotation was extremely consistent, in both direction and magnitude, from week to week.

It may be argued, however, that apomorphine-induced rotation is not due to a bilateral receptor asymmetry but is rather the result of an asymmetrical apomorphine-induced change in the dopamine metabolism of the rat's striata. Some have proposed that apomorphine's site of action is not entirely postsynaptic (Costall and Naylor, 1973; Goldstein et al., 1970; Walters and Roth, 1974). There is considerable evidence indicating that the functional activity of the nigro-striatal system is regulated by a combination of feedback control mechanisms. Through the action of one or all of these feedback control mechanisms, pharmacological agents which simulate the effect of dopamine can alter the turnover of dopamine in the striatum. However, if the mechanism of apomorphine-induced rotation were via differential dopamine metabolism in the two striata, then AMPT would be expected to decrease the rotation elicited by apomorphine. Since such a decrease did not occur in the present investigation, the proposed bilateral receptor asymmetry in the striata of normal rats is the most plausible explanation. The importance of an intrinsic nigro-striatal asymmetry has been shown previously to be most evident after unilateral lesions of the caudate nucleus (Jerussi and Glick, 1975). Rats showed significantly more rotation with apomorphine, postoperatively, if the lesion was made ipsilateral rather than contralateral to their pre-operative direction of rotation. In other words, since the two striata were not functionally equivalent, it mattered in which striatum each rat was lesioned.

Amphetamine- and Apomorphine-Induced Rotation: Pre- and/or Postsynaptic Asymmetries. The fact that rats did not necessarily rotate in the same direction with amphetamine and apomorphine suggests that both presynaptic and postsynaptic nigro-striatal asymmetries exist and that rotational behavior cannot be

explained adequately by a single neuronal mechanism. Much evidence (Dominic and Moore, 1969; Gianutsos et al., 1974; Tarsy and Baldessarini, 1974; Ungerstedt, 1971b) has indicated that procedures which reduce the concentration of a transmitter at postsynaptic sites in the central nervous system increase the sensitivity of the receptors to that transmitter (i.e. "supersensitivity" develps). Conversely, an excess of transmitter at postsynaptic sites appears to result in decreased receptor sensitivity (Overstreet et al., 1974). Perhaps variations in postsynaptic receptor sensitivity are normally related, in a reciprocal way, to variations in presynaptic activity. Thus a transmitter released from more active terminals would activate less sensitive receptors.

According to this regulatory and homeostatic model, any left-right postsynaptic asymmetry in nigrostriatal function would be compensated for by a presynaptic asymmetry in the opposite direction. Since the direction of rotation induced by amphetamine is presumably the result of both presynaptic and postsynaptic asymmetries, whereas that of apomorphine is attributed only to the postsynaptic asymmetry, rats which rotate in the same direction with each drug would be expected to have presynaptic and postsynaptic striatal asymmetries which functionally act on the same side. In animals where the direction of rotation is different for each drug, the two types of asymmetries would function opposite to each other.

L-Dopa-Induced Rotation. Since the previously tested pharmacological agents which mimicked the effect of dopamine also induced rotation in normal rats, it seemed reasonable to expect that L-Dopa, the immediate precursor of dopamine, similarly would elicit rotational behavior in normal animals. In rats with unilateral lesions or inactivation (i.e. spreading depression) of the nigro-striatal system, L-Dopa has been reported to elicit rotation (Andén et al., 1966) or body torsion (Keller et al., 1973), respectively, contralateral to the normal side. In the present investigation, L-Dopa induced significant rotation only at the highest dose (i.e. 300.0 mg/kg) in normal rats pretreated with MK 486. After the administration of L-Dopa, presynaptic levels of dopamine would be expected to be high. Thus, rotation induced by L-Dopa could possibly be attributed to bilateral differences in the normal physiological release of the newly synthesized transmitter.

Haloperidol-Induced Rotation. Haloperidol has been reported to produce catalepsy in normal rats with doses of 0.5 and 2.0 mg/kg (Costall and Olley, 1971), arrest amphetamine-induced rotation in rats with unilateral lesions of the substantia nigra at the dose of 1.0 mg/kg (Ungerstedt, 1971a), and elicit body torsion toward the intact side in unilaterally striatotomized rats in doses of 1.0 (Andén and Bedard, 1971) and 2.0 mg/kg (Andén et al., 1966). Presumably, these results are due to haloperidol's blocking properties in the 2 striata. A bilateral block would produce catalepsy, whereas a unilateral block would result in postural asymmetries. In the present investigation, haloperidol at the low dose of 0.125 mg/kg induced significant rotation in normal rats (Fig. 4), the direction of which appeared to be relatively constant throughout the test session. It is possible that low doses of haloperidol create only a partial block of the striatal receptors. However, due to the bilateral differences in striatal receptor sensitivity, previously suggested, the partial block would be bilaterally asymmetrical. That is, the two striata would not be equally blocked, and the resulting differences in nigro-striatal activity would elicit rotation. However, since there are some blockers (i.e. the anticholinergic depolarization blockers) which cause postsynaptic stimulation in low doses (Volle and Koelle, 1970), it may be that rotation elicited by haloperidol is due to such a stimulant effect of the drug.

Cholinergic Influences on Rotational Behavior. It has been proposed that the dopaminergic and cholinergic systems of the striatum are in reciprocal balance – the activity of one tends to counteract the activity of the other (Bartholini and Pletscher, 1972; Costall et al., 1972; Keller et al., 1973; Mennear, 1965). In the present investigation, doses of scopolamine, ranging from 1.0 to 200.0 mg/kg induced significant rotation in normal rats. As with amphetamine and apomorphine, the direction of scopolamine-induced rotation was consistent from week to week, and in addition, rats which rotated in one direction with amphetamine also turned to the same side with scopolamine. The consistency of direction between amphetamine- and scopolamine-induced rotation in normal rats is in agreement with the results of intracerebral injection studies. Both *d*-amphetamine and atropine produced contraversive turning after their unilateral infusion into the striatum (Costall et al., 1972; McKenzie et al., 1972; Ungerstedt et al., 1969). In contrast to the amphetamine and apomorphine consistency data, the magnitude of scopolamine-induced rotation did not remain significantly unchanged from week to week. The increase in rotations observed on Day 8 cannot be explained adequately at present.

Pharmacological agents which mimic the effect of dopamine (i.e. amantadine, *d*-amphetamine, apomorphine) cause a significant rise in striatal ACh levels (Bak et al., 1972; Glick et al., 1974; Sethy and Van Woert, 1974; respectively). Glick et al. (1974) showed that an increase in striatal ACh was inversely

related to the magnitude of rotation induced by *d*-amphetamine; presumably, the postsynaptic content of ACh was reduced. Thus, stimulating cholinergic receptors would be expected to inhibit rotation. In the present investigation, pilocarpine, a muscarinic agonist, did not induce rotation by itself and, in fact, antagonized the rotation induced by amphetamine. On the other hand, scopolamine, a muscarinic blocker, would be expected to augment amphetamine's effect unless the rotation induced by amphetamine alone was already maximal (Table 2).

Rotational Behavior: Some Theoretical Considerations. Rats with unilateral lesions of the nigro-striatal system will rotate also without drugs, when subjected to so-called stressful stimuli (e.g. tail pinching, loud noises, etc.) (Ungerstedt, 1971a), and noxious stimuli are reported to augment drug-induced rotation (McKenzie et al., 1972). In the present investigation, it appeared that the stressful environment of the test apparatus augmented the behavioral effect of the drug, and thus rotation could be elicited in the normal rat.

Numerous studies have indicated that the striatum is more than a mere component of a motor system. Apparently, the striatum is intimately associated with functions of arousal and attention (Buchwald et al., 1961; Kirby, 1973) and its integrity is necessary also for the normal expression of spatial behavior in the rat (Potegal, 1969; Zimmerberg et al., 1974). It is evident from the present investigation that left and right nigro-striatal systems of normal rats are functionally asymmetrical. If these pathways do modulate arousal then it is conceivable that an organism may have intrinsically a lower threshold for attending and turning to the source of stimulation (i.e. the orienting reflex) on one side as compared to the opposite side. Selective attention and turning to stimuli on one side could possibly engender a spatial preferences in that direction. The drug-induced rotation in normal rats may be an extreme form of spatial behavior or an exaggerated orienting reflex. Thus stimuli which create intense arousal (e.g. noxious stimuli, pharmacological stimulation of the striatal receptors) overload the functionally asymmetrical nigro-striatal pathways and reflexively turn the animal in a preferred or dominant direction.

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