PHARMACOLOGICALLY INDUCED BEHAVIORAL CORRELATES OF NIGRO-STRIATAL FUNCTION

by

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Abstract

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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. Amphetamine-induced rotation was significantly antagonized by alpha-methyl-p-tyrosine and haloperidol, but not by diethyldithiocarbamate. On the other hand, the magnitude of rotation elicited by apomorphine was unaffected by alpha-methyl-p-tyrosine. For each rat, the direction of rotation was consistent from week to week when tested with amphetamine, apomorphine, or scopolamine. In addition the magnitude of rotation was significantly correlated and not significantly changed by the weekly administration of amphetamine or apomorphine. Rats did not necessarily rotate to the same direction with high and low doses of amphetamine, or amphetamine and apomorphine administered a week apart from each other. These results suggested that normal rats may have a bilateral asymmetrical content of striatal dopamine as

well as an asymmetrical complement of striatal dopamine receptors. The importance of a nigro-striatal asymmetry was most evident after unilateral lesions of the caudate nucleus, for rats rotated more post-operatively if the lesion was made ipsilateral rather than contralateral to the animals' pre-operative direction of rotation. Dopaminergic-cholinergic interactions were evident, since pilocarpine antagonized amphetamine-induced rotation whereas scopolamine did not, and scopolamine elicited rotation in the same direction as that induced by amphetamine.

Bilateral regional neurochemistry was performed on rats injected with various doses of d-amphetamine and tested for rotation. The only chemical measure of significance was the differences in striatal dopamine content, ipsilateral and contralateral to the direction of rotation induced by the drug. Since amphetamine was not found to be unequally distributed to the two sides of the brain, it appeared that the neurochemical substrate for rotation in normal rats was the difference in striatal dopamine content.

The theoretical implications of the results were discussed in relation to arousal, spatial behavior, and handedness, and how these functions could have evolved from the same bilateral striatal asymmetry.

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LIST OF ABBREVIATIONS

ACh.....acetylcholine
AChE.....acetylcholinesterase
AMPT.....alpha-methyl-para-tyrosine
CAT.....cholinacetyl transferase
CM.....centromedian
COMT.....catechol-O-methyl transferase
DDC.....diethyldithiocarbamate
DOPA.....dihydroxyphenylalanine
DOPAC.....3, 4-dihydroxyphenylacetic acid
GABA.....gamma-aminobutyric acid
HVA.....homovanillic acid
MAO.....monoamine oxidase
MHPG.....3-methoxy-4-hydroxy-phenylethylene glycol
NCE cells.....caudate neurons which are excited by electrical stimulation
NCI cells.....caudate neurons which are depressed by electrical stimulation
NISTS.....nigro-striatal system
QNB.....quinuclidinyl benzilate
SN.....substantia nigra
VA.....ventral anterior

INTRODUCTION

Anatomy

The nigro-striatal system (NISTS) of the rat brain has been described as a bilateral monoaminergic bundle of nerve fibers, which originate from cell bodies in the pars compacta of the substantia nigra (designated as A9 by Dahlstrom & Fuxe, 1964), pass through the lateral hypothalamus without synapsing, ascend rostromedially via the crus cerebri and internal capsule, and terminate predominantly in the neostriatum or the caudate-putamen complex (Bedard & Larochelle, 1973; Dahlstrom & Fuxe, 1964; Ungerstedt, 1971a). The caudate-putamen complex (hereafter referred to as the striatum or caudate) comprises a large portion (about 7 mm rostro-caudally) of the telencephalon of the rat brain. On the other hand, the rostro-caudal extent of the substantia nigra (SN) in the mesencephalon is only about 2 to 3 mm (Crow, 1971; Pelligrino & Cushman, 1967).

Putative neurochemical transmitters within the striatum

Although there are high concentrations of both monoamines 5-hydroxytryptamine (serotonin) and 3-hydroxytyramine (dopamine), biochemical and histochemical fluorescence techniques coupled with degeneration studies indicate that dopamine is the putative neurotransmitter of the ascending NISTS. Striatal

dopamine content is depleted after a lesion of the pars compacta of the SN. However, serotonin content is virtually unchanged after a similar lesion of the nigra (Marsden, Broch, & Guldberg, 1972; Marsden & Guldberg, 1973). The relatively high content of serotonin (0.35 µg/g--Bak, Hassler, Kim, & Kataoka, 1972; 0.77 µg/g--Broch & Marsden, 1972) in the caudate nucleus of the rat appears to originate from the presynaptic terminals of axons which have their cell bodies in the raphe nuclei of the brainstem (Dahlstrom & Fuxe, 1964; Fuxe, Hokfelt, & Ungerstedt, 1968). After lesions of the raphe nuclei, serotonin is depleted by about 60-75%, whereas the dopamine content is unchanged (Marsden et al., 1972; Marsden & Guldberg, 1973).

Ungerstedt (1971a), using a serial lesioning technique or injection of alpha-methyl-norepinephrine to enhance detection of the NISTS, has reported the intense greenish fluorescence, indicative of catecholamines, in the head of the caudate nucleus all the way back to the SN. Neurochemical analyses of the caudate reveal the presence of dopamine to norepinephrine in the ratio of 100 to 1 (10.0 µg/g to 0.1 µg/g) and incubation of striatal slices with ^{3}H-tyrosine produces large amounts of ^{3}H-dopamine but little or not ^{3}H-norepinephrine (Hornykiewicz, 1966). Thus it seems reasonable to conclude that the main contributor to the NISTS fluorescence and therefore the neurochemical transmitter of that system is dopamine and not norepinephrine.

More recently, high concentrations (4.5 µg/g--Bak et al., 1972) of gamma-aminobutyric acid (GABA) have been reported to

be located in the rat striatum (Bak et al., 1972; Kim, Bak, Hassler, & Okada, 1971; Okada, Hassler, Kim, Bak, & Hassler, 1971). Although it is possible that GABA may be the neurotransmitter of the fibers coursing from the caudate to the nigra, the inhibitory amino acid has not been implicated as the mediator of synaptic transmission in the NISTS (Hattori, McGeer, Fibiger, & McGeer, 1973; Precht & Yoshida, 1971).

Although the caudate is rich in the monoamines serotonin, dopamine, and the amino acid GABA, it also contains high levels of acetylcholine (ACh) (about 6.5 μg/g--Bak et al., 1972), acetylcholinesterase (AChE), and cholineacetyl transferase (CAT) (Andén, Dahlstrom, Fuxe, & Larsson, 1966; McGeer, McGeer, Fibiger, & Wickson, 1971). It is possible that ACh fibers may ascend without decussating in the crus cerebri and course through the internal capsule before terminating in the ipsilateral striatum (Anden et al., 1966), since since unilateral lesions in the more roatral mesencephalon have been reported to cause a significant decrease in the AChE content of the ipsilateral caudate (Shute & Lewis, 1963). However, more recent evidence seems to indicate that the striatal ACh content is of an intrinsic nature. Since various lesions of the cerebral cortex, thalamus, globus pallidus, or ventral tegmentum produced no consistent significant decreases in AChE or CAT, McGeer et al. (1971) concluded that the major cholinergic activity in the striatum was associated with those neurons which did not have efferents outside the caudate. Thus the cholinergic influences on striatal function appear to be largely of an intrinsic nature. While striatal

dopamine is contained in the presynaptic terminals of the NISTS, ACh appears to be located within the neuronal cells of the caudate itself. However, due to the lack of a staining technique specific for ACh, the controversy of intrinsic or extrinsic striatal ACh cannot be verified histochemically.

Ultrastructure of the striatum: localization of dopamine

Ultrastructure reveals that the highest content of dopamine is contained in the presynaptic terminals of the striatum, while lesser amounts of the presumed transmitter are found in the cell bodies of the SN (Dahlstrom & Fuxe, 1964; Fuxe, Hokfelt, & Nilsson, 1964). The cell population of the caudate nucleus of the rat is rather heterogenous consisting of neuronal cells of various sizes (ranging from 10 to 20 μ in diameter) and shapes (Mori, 1966; Willis & Grossman, 1973), which are arranged in clumps, rather than in a homogenous organized fashion, and immersed in a thick mesh-like neuropil (Marco, Copack, Edelson, & Gilman, 1973; Mori, 1966; Tennyson, & Marco, 1973). The small cells of the striatum have short, thin, axon fibers, most of which probably terminate within the caudate nucleus itself (Mori, 1966). According to Fuxe et al., (1964) some of the nerve terminals are so fine (about 0.1 μ) that they are nearly below the resolving power of the fluorescence microscope. On the other hand, the large striatal cells, which appear to be Golgi type I and Golgi type II neurons (Mori, 1966), may be better suited than the small cells for synaptic contact outside the striatum, especially the longer axons of the Golgi type I cells. Synaptic

organization within the caudate typically consists of a single axon making contact with many dendrites or what has been described as "en passant" terminals (Mori, 1966; Tennyson & Marco, 1973).

Although at least five different types of synaptic vesicles have been identified in the striatum of the rat (Mori, 1966), it cannot be stated conclusively which of these vesicles, if any, contain the neurochemical dopamine. In the periphery, catecholamine containing synaptic vesicles contain an electron dense core (Bloom & Aghajanian, 1968; Hokfelt, 1968). In the caudate, however, the small dense core vesicles have not been shown to contain dopamine, and the large granular synaptic vesicles are too sparse to account for the high content of dopamine in that nucleus (Bloom & Aghajanian, 1968). Although large granular vesicles are still present after surgically isolating the caudate of the cat, fluorescence indicative of catecholamines is no longer visible (Tennyson & Marco, 1973). According to Philippu and Heyd (1970), most (54%) of the dopamine in the striatum and pallidum (pig) is not stored in subcellular particles, but is found free in the cytoplasm.

Electrically stimulated and pharmacologically induced release of neurochemicals from the striatum

Both monoamines (i.e. dopamine and serotonin) and ACh can be released from the striatum by perturbations of the appropriate neuroanatomical structures. Serotonin could be released from the caudate nucleus by stimulating the nucleus linearis intermedius or rostralis, but stimulation of other

structures outside the two linear nuclei, including the SN (Portig & Vogt, 1969), did not have a similar effect (Holman & Vogt, 1972). On the other hand, electrical stimulation of the nigra, and thus presumably the nigro-striatal bundle, resulted in the release of both dopamine and ACh from the striatum of the cat (McLennan, 1965; Portig & Vogt, 1969).

Pharmacological agents, as well as electrical stimulation of the NISTS, can release dopamine from the striatum. Catecholamines can be released from both peripheral nerve terminals and presynaptic endings of the central nervous system by certain phenylethylamines (e.g. amphetamine) (Axelrod, 1970; Burn & Rand, 1958; Carlsson, Fuxe, Hamberger, & Lindqvist, 1966; Carr & Moore, 1969; Glowinski & Axelrod, 1965; Hanson, 1967; Randrup & Munkvad, 1966; Sulser, Owens, Norvich, & Dingell, 1967; Trendelenburg, Muskus, Fleming, & de la Sierra, 1962). Von Voigtlander and Moore (1973) have demonstrated the efflux of ^{3}H-dopamine from the cat caudate after perfusing the cerebroventricular system with amphetamine, amantadine, or tyramine. The release of the amine was dose related and concomitant low frequency electrical stimulation of the nigra potentiated the drug-induced release. Amphetamine's effect was reduced, however, in animals with chronic lesions of the NISTS. Amphetamine-evoked release of dopamine from the striatum was also evident in the rat, by the accumulation of O-methylated catecholamines after inhibition of monoamine oxidase (MAO) (Anden & Svensson, 1973).

Neurophysiological cholinergic-dopaminergic interactions within the striatum

Although it is believed that there is a normal functional balance between the dopaminergic and cholinergic influences in the caudate (Andén et al., 1966), this interaction has not been elucidated electrophysiologically or pharmacologically. McLennan (1964) has shown that low frequency electrical stimulation of the ventral anterior (VA) nucleus of the thalamus (cat), will increase the resting release of ACh. Similar stimulation of the centromedian (CM) thalamic nucleus, anterior sigmoid gyrus, or the SN will increase dopamine's release from the striatum (McLennan, 1964; McLennan, 1965). The electrophysiological effects of VA stimulation could be mimicked by iontophoretic application of ACh to single caudate neurons (McLennan & York, 1966). Some cells were excited by ACh and/or VA stimulation, while others were depressed. Cholinergic effects could be blocked by systemic administration of atropine; methacholine, but not tetramethylammonium or nicotine, could mimic the effect of ACh. Thus it appears that the cholinergic influence in the caudate is primarily muscarinic and can be either excitatory or inhibitory. These inhibitory and excitatory cells were found in distinct portions of the caudate; cholinergic excitatory cells appeared to form a definite lamina surrounding other cells which responded with a decreased rate of firing after cholinergic stimulation.

When dopamine was iontophoretically applied to single units of the cat caudate, the predominant effect was supression of firing; even responses evoked by electrical stimulation of the nigra were depressed by the application of dopamine. Unit responses elicited by dopamine could be blocked by prior

iontophoretic injection of phenoxybenzamine, but not by the application of dichloroisoproterenol. Thus it seems reasonable to suggest that dopaminergic effects in the caudate are mediated at a site which is characteristic, pharmacologically, of an alpha-receptor. Whereas the cholinoceptive cells appear to have an ordered distribution within the caudate, no such organization was found for cells which responded to dopamine. Some striatal cells, responded by excitation or depression of firing after the application of both ACh or dopamine. That is in any particular cell both neurochemicals could similarly increase or supress firing, or have completely opposite effects (McLennan & York, 1967). According to Buchwald, Price, Vernon, and Hull (1973), the major input to the caudate is excitatory; they suggest that excitation impinging on inhibitory interneurons (presumably cholingergic) accounts for the depression of firing within the caudate.

Feltz and Albe-Fessard (1972) demonstrated two types of caudate cells upon stimulating the SN. Spontaneously active caudate cells (NCI cells) were depressed during nigral stimulation, whereas other cells (NCE cells) were induced to fire after similar electrical stimulation. These excitatory NCE cells appear to have the approximate location, in the medial two-thirds of the caudate nucleus as the cholinoceptive inhibitory cells described by McLennan and York (1966). Is it possible that these cells receive excitatory dopaminergic input from the nigra and inhibitory cholinergic afferents from the VA nucleus of the thalamus? Perhaps all caudate afferents are excitatory as Buchwald et al. (1973) maintains, and the firing

of the NCE cells, described by Feltz and Albe-Fessard (1972), is depressed by striatal cholinergic interneurons receiving afferents from the VA nucleus of the thalamus. Since the monosynaptically excited NCE neurons were the smallest cells found in the caudate and their connections were primarily within that nucleus, Feltz and Albe-Fessard (1972) concluded that the NCE cells themselves were the inhibitory interneurons. These interneurons presumably modulate the activity of the spontaneously active NCI cells. From the preceeding discussion it is apparant that the cholinergic-dopaminergic interactions within the caudate nucleus itself are complex and still the subject of considerable speculation.

Neurophysiological cholinergic-dopaminergic interactions outside the caudate

Cholinergic influences appear to be important in determining the activity of the NISTS as a whole (Bartholini & Pletscher, 1972). A feedback loop from the caudate nucleus to the SN, which modulates unit activity in the latter, has been postulated (Bunney, Walters, Roth, & Aghajanian, 1973b; Costall, Naylor, & Olley, 1972; McNair, Sutin, & Tsubokawa, 1972; Oliver, Parent, Simard, & Poirier, 1970). These strionigral fibers have been reported to be rich in AChE (Oliver et al., 1970). The SN, like the caudate, is rich also in GABA (Bak et al., 1972; Kim et al., 1971; Okada et al., 1971), and it has been proposed (Precht & Yoshida, 1971) that the amino acid may mediate neurochemical transmission in the strionigral pathway. However, more recent evidence suggests that the high levels of nigral GABA are localized primarily in the

presynaptic terminals of pallido-nigral fibers (Hattori et al., 1973).

McNair et al., (1972), and Precht and Yoshida (1971) demonstrated that single shock stimuli applied to the caudate nucleus of the cat orthodromically supressed the firing of nigral cells even after the caudate stimulation had ceased. Pharmacological agents which mimic the electrical stimulation of the caudate, either by release of dopamine and subsequent blockage of its reuptake (e.g. amphetamine) or direct stimulation of dopaminergic receptors (e.g. apomorphine), also supress the firing of units in the pars compacta of the nigra (Bunney, Aghajanian, & Roth, 1973a; Bunney et al., 1973b). Small doses of intravenously administered d-amphetamine or apomorphine were sufficient to depress the firing rate of the dopaminergic cells of the SN. Dihydroxyphenylalanine (L-DOPA) usually had no effect on the firing of nigral cells. However, when the rat was pretreated with an inhibitor of peripherial DOPA decarboxylase, there was the expected depression of nigral firing (Bunney et al., 1973a). Presumably the peripheral decarboxylase inhibitor allowed more L-DOPA to enter the brain where it then could be decarboxylated to form dopamine. On the other hand, drugs which decrease dopamine transmission by blocking the recptor (e.g. haloperidol, chlorpromazine), increase rather than decrease the rate of firing of SN cells. Not only did the intravenous administration of the antipsychotic phenothiazines and haloperidol increase the rate of firing of nigral cells, but there was a reversal of the amphetamine-induced supression of firing. The administration of promethazine,

however, a phenothiazine with no antipsychotic activity, was without effect (Bunney et al., 1973b). Thus it appears that an increase in the neuronal activity of the striatum, will supress the firing of nigral cells by means of a cholinergic feedback strio-nigral loop, while decreases in caudate activity will have the opposite effect on nigral units.

Feedback mechanisms regulating dopamine metabolism

The electrical responses occurring in the SN after the administration of various drugs may be indicative of neurochemical changes taking place in the NISTS. Apparently, the strio-nigral feedback loop is an effective means of regulating dopamine synthesis and catabolism. Bunney et al., (1973b) has demonstrated that d-amphetamine, which decreases the rate of firing of nigral units, also decreases dopamine turnover in the corpus striatum as measured by the accumulation of 3,4-dihydroxyphenylacetic acid (DOPAC). Chlorpromazine, on the other hand, increases the striatal DOPAC content. Homovanillic acid (HVA) also has been reported to increase after the administration of haloperidol (Andén, 1970; Goldstein, Anagnoste, & Shirron, 1973). The results of Walters, Roth, and Aghajanian (1973) indicate that procedures which decrease SN firing and thus reduce impulse transmission in the NISTS (e. g. axonomy, administration of gamma-butyrolactone temporily increase dopamine content in the rat striatum; an increase in dopamine synthesis is associated with a decrease in neuronal activity of the NISTS. These data are difficult to reconcile with the fact that haloperidol increases both SN firing and dopamine synthesis. Again cholinergic involvement

is implicated, since the haloperidol-induced decrease in striatal dopamine after alpha-methyl-p-tyrosine pretreatment (Andén & Bedard, 1971), and the neuroleptic-induced rise in HVA (Bartholini & Pletscher, 1972) were both counteracted by the intraperitoneal administration of anticholinergics.

Although the changes in dopamine metabolism can be explained by a neuronal feedback loop, the actual involvement of this mechanism is still somewhat uncertain. It appears that additional feedback regulation of dopamine synthesis can occur at the site of the dopmine receptor itself, since various pharmacological agents can effect changes in dopamine metabolism without concomitant impulse transmission through the strio-nigral pathway or the NISTS. After inhibiting DOPA decarboxylase and unilaterally sectioning the nigro-striatal bundle of the rat, there is a greater accumulation of DOPA on the lesioned side than on the intact side (Kehr, Carlsson, Lindqvist, Magnusson, & Atack, 1972). This observation is in agreement with a neuronal feedback interpretation of the regulation of dopamine metabolism. Reduced impulse transmission on the lesioned side would signal compensatory mechanisms in the striatum to increase dopamine synthesis. However, the administration of apomorphine to such lesioned rats prevented the striatal accumulation of DOPA on both sides, and this effect was significantly reversed by haloperidol (Kehr et al., 1972). In addition, Bedard and Larochelle (1973) reported that sectioning the cholinesterase staining strio-nigral fibers, without damaging the nigro-striatal bundle, did not prevent the increase of striatal HVA in rats treated with

haloperidol. These results would tend to minimize the importance of an extra-striatal feedback control of dopamine metabolism. Thus Kehr et al. (1972) and Goldstein et al. (1973) agree that striatal tyrosine hydroxylase activity is regulated by the dopamine receptors themselves which are instrinsic to the caudate.

Although both neuronal feedback and receptor mediated mechanisms for the control of dopamine synthesis have been postulated, there is also considerable evidence to indicate that dopamine levels are regulated neurochemically by a third intraneuronal mechanism--end-product inhibition (Harris & Roth, 1971; Javoy, Agid, Bouvet, & Glowinski, 1972). After inhibiting MAO *in vivo*, striatal dopamine content rapidly increased, in a linear fashion within the first ten minutes, and eventually reached about 145% of its control value. Since there was no increase in the metabolite 3-methoxytyramine, it was concluded that the catabolism of the amine had not increased, but rather its synthesis had been markedly decreased at this time (Javoy et al., 1972). *In vitro* experiments have demonstrated that dopamine concentrations as low as 10^{-6} M inhibit, from 25% (Harris & Roth, 1971) to 43% (Javoy et al., 1972) of control values, the conversion of ^{3}H-tyrosine to ^{3}H-DOPA in slices of the rat striatum. Drugs which block the neuronal reuptake of catecholamines (i.e. cocaine, benztropine) could partially prevent the catecholamine-induced decrease in the conversion of labeled tyrosine to DOPA, even though these drugs had no effect by themselves on the activity of tyrosine hydroxylase (Harris & Roth, 1971; Javoy et al., 1972). It has been

suggested that dopamine regulates the tyrosine-DOPA conversion by inhibiting tyrosine hydroxylase uncompetitively and the pteredine cofactor competitively (Ikeda, Fahien, & Udenfriend, 1966). However, end-product inhibition cannot account for the observation that apomorphine inhibits the conversion of tyrosine to DOPA in striatal slices, more effectively than the dopaminergic agonist inhibits the _in vitro_ activity of tyrosine hydroxylase (Goldstein, Freeman, & Backstrom, 1970). Thus it appears that there may be at least three feedback mechanisms which regulate dopamine metabolism _in vivo_: 1) a descending cholinergic (or possibly "GABAergic") neuronal feedback loop from the caudate to the SN which regulates unit activity of the nigra (subsequent impulse transmission through the NISTS effects changes in striatal dopamine metabolism); 2) a receptor-mediated feedback mechanism which controls the transmitter's metabolism via dopaminergic activity postsynaptically or within the synaptic cleft; 3) a neurochemical end product inhibition of dopamine on tyrosine hydroxylase activity.

Nigro-striatal function: clinical studies

In a larger context, the normal functioning of the extrapyramidal motor system is dependent upon an intact NISTS (Jung & Hassler, 1960). From clinical studies it is evident that perturbations of the NISTS can lead to severe motor disturbances. For example, lesions of the putamen result in a dystonic syndrome which resembles proximal athetosis. Destruction of the striatum (especially the small cells) or combined lesions of both striatum and globus pallidus involve more distal muscle

groups with the resulting symptoms characteristic of chorea and athetosis, respectively (Jung & Hassler, 1960; Willis & Grossman, 1973). The ablated striatum is also thought to be the neuroanatomical substrate for the myoclonic stereotypic movements manifested clinically. On the other hand, parkinsonism which is characterized by tremor at rest, rigidity, and akinesia, is associated with destruction of the melanin pigmented cells in the SN with resulting degeneration of the nigro-striatal tract (Hornykiewicz, 1966; Jung & Hassler, 1960; Willis & Grossman, 1973). Unilateral electrical stimulation of the pallidum in man, consistently evokes a tendency, in most patients, to fix their gaze in the contralateral direction (Jung & Hassler, 1960).

Nigro-striatal function: animal studies

From animal studies, the most consistent behavioral phenomenon obtained during unilateral electrical stimulation of the caudate nucleus is contraversive turning (i.e. turning away from the stimulating electrode) (Forman & Ward, 1957; Hendley & Hodes, 1953; Jung & Hassler, 1960). Forman and Ward (1957) were able to show that contraversive turning of the head and body were somatotopically organized within the caudate nucleus of the cat, and the production of these movements was not dependent upon an intact motor cortex. On the other hand, contraversive movement was dependent upon an intact connection between the caudate and the SN (Hendley & Hodes, 1953), and nigral stimulation evoked turning behavior similar to that produced by caudate stimulation (Arbuthnott & Crow, 1971; York,

1973).

Large bilateral lesions of the cat caudate produced a motor disturbance termed "obstinate progression", that is the animal had a tendency to walk straight ahead despite obstacles placed in its path. On the other hand, subtotal unilateral caudate lesions evoked motor asymmetries similar to the behavior elicited with electrical stimulation (i.e contraversive turning); circling movements occurred to the side of the lesion (Jung & Hassler, 1960).

The nigro-striatal system and rotational behavior

The neuroanatomical substrates for the initiation of contraversive turning movements have been proposed to be manifold and found in many areas of the brain (Jung & Hassler, 1960). Although the data are controversial, one such system originates in the anterior nucleus of the thalamus, sends efferents to the cingulate gyrus which then terminate in the pallidum. Another system begins in the caudate nucleus; it too ends in the globus pallidus. Apparently, pallidal efferents cross the midline in the mesencephalon and eventually communicate with the reticulospinal tract which in turn conveys these efferent impulses into motor output. Involvement of the reticulospinal tract, in the mediation of circling, has also been suggested more recently by Bak et al. (1972) and York (1973). However, substantial evidence has now accumulated which implicates the ascending dopaminergic NISTS as the neuroanatomical substrate for the initiation of circling or rotational behavior in the rat (Andén, 1970; Andén et al., 1966; Arbuthnott & Crow, 1971; Costall et al., 1972; Crow, 1971;

Ungerstedt, 1971b; Ungerstedt & Arbuthnott, 1970).

Rats with unilateral lesions of the striatum or the ventromedial tegmental area of the mesencephalon exhibit a postural asymmetry upon recovering from the operation, which consists of turning ipsilateral to the lesion (Andén, et. al., 1966; Ungerstedt, 1971b). After the administration of amphetamines (e.g. 1-5 mg/kg of d-amphetamine) to such lesioned rats, they vigorously rotate or turn in circles towards the operated side (Andén, et al., 1966; Christie & Crow, 1971, 1973; Crow, 1971; Ungerstedt, 1971b; Ungerstedt & Arbuthnott, 1970). Crow (1971) has demonstrated that unilateral lesions of the mesencephalon, only encroaching upon the SN or interrupting the medial nigrostriatal bundle in the crus cerebri, will result in rotation after the administration of methamphetamine. It should be noted that the interpeduncular nucleus (designated as A10 by Dahlstrom & Fuxe, 1964), which contains the cell bodies of the mesolimbic dopaminergic system (Ungerstedt, 1971a), lies close to the SN in the midbrain; stimulation of the area around A10 produces some of the behavioral components of stereotypy in the rat (i.e. licking, sniffing, gnawing) (Crow, 1971). However, rotational behavior is not elicited after electrical stimulation of the interpeduncular nucleus, and lesions of the SN alone are sufficient to produce circling under the appropriate pharmacological conditions (Arbuthnott & Crow, 1971). Glick and Greenstein (1973) reported amphetamine-induced rotation in rats with unilateral frontal lesions. However, their interpretation is not inconsistent with the idea that the NISTS is the substrate for rotation, since these investigators considered

the frontal cortex as a modulator of nigro-striatal function.

The neurochemical basis of rotation

The neurochemical basis of rotational behavior has been attributed to a functional imbalance between the NISTS ipsilateral and contralateral to the lesion (Christie & Crow, 1971; Ungerstedt, 1971b; Ungerstedt & Arbuthnott, 1970). Amphetamine presumably stimulates the intact system, i.e. releases dopamine which further enhances the bilateral imbalance and causes the rat to rotate. Thus after the administration of amphetamine, the animal rotates toward the side containing the lowest dopamine content.

Although there are other neurotransmitters in the striatum, only changes in dopamine content and/or metabolism have been correlated with rotational behavior. Alpha-methyl-p-tyrosine which inhibits tyrosine hydroxylase (Spector, Sjoerdsma, & Udenfriend, 1965; Weissman, Koe, & Tenen, 1966) and subsequent catecholamine synthesis, markedly reduces or completely abolishes amphetamine-induced rotation in rats with unilateral lesions of the SN (Christie & Crow, 1971; Ungerstedt, 1971b). On the other hand, the same rats pretreated with FLA63 [bis-(1-methyl-4-homopiperazinyl-thiocarbonyl)-disulphide], an inhibitor of dopamine-beta-hydroxylase (Corrodi, Fuxe, Hamberger, & Ljungdahl, 1970) which selectively blocks norepinephrine synthesis, showed no decrease in rotation after the administration of amphetamine (Christie & Crow, 1971; Ungerstedt, 1971b). The dopaminergic agonist, apomorphine, causes rotation in rats with unilateral lesions of the NISTS

(Andén, 1970; Ungerstedt, 1971b), and stereotypic behaviors in normal animals (Andén, Strombom, & Svensson, 1973; Ernst, 1965, 1967). However, rotation or stereotypy has been reported not to occur after the administration of clonidine (Andén et al., 1973)--a drug which purportively stimulates noradrenergic receptors (Andén, 1970; Andén et al., 1973).

Both chlorpromazine and haloperidol will block drug-induced rotation in rats with unilateral nigro-striatal lesions (Christie & Crow, 1971; Ungerstedt, 1971b). Apparently haloperidol has a greater efficacy in preventing rotation, since the butyrophenone has a higher dopaminergic to noreadrenergic blocking ratio than chlorpromazine (Anden, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970). When haloperidol or chlorpromazine alone is administered to rats with unilateral nigro-striatal lesions, a bending of the body to the unoperated side is induced. This postural asymmetry is not observed after the administration of the alpha blocker, phenoxybenzamine, or the beta blocker, propranolol (Andén, 1970; Andén et al., 1970; Andén et al., 1966).

The case for dopamine/nigro-striatal involvement in rotational behavior is further strengthened by investigations using intracerebral injection of various pharmacological agents. Contralateral circling has been observed after the unilateral injection of dopamine, apomorphine, (Ungerstedt, Butcher, Butcher, Andén, & Fuxe, 1969) or amphetamine (McKenzie, Gordon, & Vilk, 1972) directly into the neostriatum. On the other hand, ipsiversive rotation is induced by the unilateral intrastriatal application of haloperidol (Costall et al., 1972) or

chlorpromazine (Ungerstedt et al., 1969).

On the basis of the preceding evidence, it is apparent that dopamine and not norepinephrine is the neurochemical mediator of rotational behavior in rats. As mentioned previously, serotonin content in the striatum is high, and the indolamine can be depleted selectively by lesions of the raphe nuclei (Marsden et al., 1972). However, amphetamine administered to rats with raphe lesions, did not elicit rotation, and the magnitude of amphetamine-induced rotation in rats with unilateral SN lesions, remained unchanged even after subsequent lesions of the raphe nuclei (Marsden & Guldberg, 1973). Rotation is observed when L-DOPA is administered to unilaterally striatotomized rats which have been pretreated with an inhibitor of MAO. However, rotation does not occur under the same conditions, if 5-hydroxytryptophan, the immediate precursor of serotonin, is substituted for L-DOPA (Andén et al., 1966).

Cholinergic influences on rotational behavior

The preceding evidence indicates that monoamines other than dopamine are not involved in drug-induced rotation in rats with unilateral lesions of the NISTS. However, 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 drugs arecoline (Costall et al., 1972) or neostigmine (McKenzie et al., 1972) are administered by the same technique, rotation in the opposite direction

is observed. Although the systemic administration of scopolamine itself has not been reported to elicit rotation in unilaterally striatotomized rats, the anticholinergic does antagonize the haloperidol-induced turning of the head and tail to the unoperated side (Andén & Bedard, 1971). There are data indicating that the cholinergic influences on rotational behavior are mediated via muscarinic receptors. Apomorphine-induced rotation in rats with functional inactivation of one striatum, by the application of 25% KCl (i.e. spreading depression), is antagonized by cholinergic drugs such as arecoline and oxotremorine, but not by small doses of nicotine. Atropine, on the other hand, can block this action of arecoline (Keller, Bartholini, & Pletscher, 1973). 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.

The present investigation

The present study was begun as a result of the unexpected observation that the sytemic administration of high doses (i.e. greater than 5.0 mg/kg) of d-amphetamine could elicit rotation in normal unoperated rats. This finding suggested that perhaps rats may normally have a functional imbalance between the two NISTS. The possibility was tested by exploring the specificity of the rotation phenomenon with respect to pharmacological agents used in the previous lesion studies.

BEHAVIORAL METHODS

General

Female Sprague-Dawley rats about three months of age and ranging in weight from approximately 200 to 300 grams were used in all the experiments. The animals were individually tested for rotation in one of two identical modified "rotometers" (Glick & Greenstein, 1973) described initially by Ungerstedt and Arbuthnott (1970). The apparatus consisted of a white opaque Plexiglass sphere, 12 inches 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 indicated left or right turns. Generally, 15 minutes after the rat had been placed in the apparatus, it was injected intraperitoneally (i.p.) with the test drug or its diluent. Rotations were automatically recorded on a print-out counter, at five minute intervals, for the 15 minutes before and 60 minutes after injection. Rotations to the left or right during the pre- and post-injection periods were separately totalled and net rotations (e.g. rotations to the left minus rotations to the right) 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 ten per hour) were selected for repeated testing.

The pharmacological agent was usually dissolved in physiological (0.9%) saline, and the volume of injection was 1.0 ml/kg unless otherwise stated. All doses are expressed as milligrams per kilogram (mg/kg). 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); diethyldithiocarbmate (DDC); beta-(3,4-dihydroxyphenyl)-alpha-hydrazino-alpha-methyl propionic acid (MK 486--Merck, Sharp & Dome); haloperidol.

Dose-Response Relationships

Unless otherwise specified, all dose-response relationships were generated using six rats for each dose of the drug under investigation. Rotationsto the left or right during the pre- 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.

Amphetamine dose-response

The following doses of amphetamine were used; 0.0 (saline), 0.625, 1.0, 1.25, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 25.0 mg/kg.

Amphetamine and AMPT dose-response interactions. Rats were pretreated with 150.0 mg/kg of AMPT 135 min. prior to

the injection of one of the following doses of amphetamine: 0.0 (saline), 1.0, 2.5, 5.0, 15.0, 25.0 mg/kg.

Amphetamine and haloperidol dose-response interactions. Rats were pretreated with 1.0 mg/kg of haloperidol 60 minutes prior to the injection of one of the following doses of amphetamine: 0.0 (saline), 1.0, 2.5, 5.0, 15.0, 25.0 mg/kg.

Amphetamine and DDC dose-response interactions. Rats were pretreated with 200.0 mg/kg of DDC 45 minutes prior to the injection of one of the following doses of amphetamine: 0.0 (saline), 1.0, 2.5, 5.0, 15.0, 25.0 mg/kg.

Apomorphine dose-response

Distilled water was used as the diluent for the following doses of apomorphine: 0.0 (distilled water), 0.5, 1.0, 2.5, 5.0, 10.0 25.0, 50.0 mg/kg. The volume of injection was 2.0 ml/kg.

L-DOPA dose-response

Rats were pretreated with 150.0 mg/kg of MK 486 dissolved in saline (adjusted to pH 1) 30 minutes prior to the injection of a saline solution (pH 1) or one of the following doses of L-DOPA (4.0 ml/kg; pH 1): 0.0 (saline) 75.0, 150.0, 300.0 mg/kg.

Haloperidol dose-response

Rats were injected with a fine suspension of haloperidol in saline 45 minutes prior to their placement in the rotometer. After 15 minutes in the apparatus, rotations were recorded for

an additional 60 minutes. The following doses of haloperidol were used: 0.0 (saline), 0.0625, 0.125, 0.25, 0.5, 1.0 mg/kg.

Scopolamine dose-response

The following doses of scopolamine were administered: 0.0 (saline), 1.0, 10.0, 100.0, 200.0 mg/kg.

Pilocarpine dose-response

The following doses (2 rats/dose) of pilocarpine were used: 0.0 (saline), 1.0, 10.0, 100.0, 200.0 mg/kg.

Consistency of Pharmacologically Induced Rotation: Directional and Quantitative Correlations

Rats were tested twice with the drug under investigation, and an interval of one week separated the two testing sessions (i.e. Day 1 and Day 8).

Amphetamine

Fifteen rats were tested with a dose of 1.0 mg/kg of amphetamine.

Apomorphine

Nineteen rats were tested with a dose of 10.0 mg/kg of apomorphine dissolved in distilled water.

Scopolamine

Thirteen rats were tested with a dose of 1.0 mg/kg of scopolamine.

Directional Consistency between Drugs which Induce Rotation

Rats were tested for rotation and an interval of one week

separated the two testing sessions (i.e. Day 1 and Day 8).

Amphetamine (low dose vs. high dose)

Nine rats were tested initially with 1.0 mg/kg of an amphetamine. The following week, the same animals were tested with a dose of 20.0 mg/kg of amphetamine.

Amphetamine vs. apomorphine

Fourteen rats were tested initially with 10.0 mg/kg of apomorphine dissolved in distilled water. The following week, the same animals were tested with a dose of 1.0 mg/kg of amphetamine.

Amphetamine vs. scopolamine

Seven rats were tested initially with 1.0 mg/kg of scopolamine. The following week, the same animals were tested with a dose of 1.0 mg/kg of amphetamine.

Pharmacological Interactions: Effects on Rotational Behavior

Rats were tested twice for rotation and an interval of one week separated the two testing sessions (Day 1 and Day 8).

Amphetamine and AMPT interactions

Six rats were tested initially with 1.0 mg/kg of amphetamine, The following week, the same animals were pretreated with 150.0 mg/kg of AMPT 135 min. prior to their retest with 1.0 mg/kg of amphetamine.

Amphetamine and scopolamine interactions

Eight rats were tested initially with 1.0 mg/kg of

amphetamine. The following week, the same animals were tested with the combination of 1.0 mg/kg of amphetamine plus 1.0 mg/kg of scopolamine.

Amphetamine and pilocarpine interactions

Eight rats were tested initially with 1.0 mg/kg of amphetamine. The following week, the same animals were tested with the combination of 1.0 mg/kg of amphetamine plus 1.0 mg/kg of pilocarpine.

Apomorphine and AMPT interactions

Six rats were tested initially with 10.0 mg/kg of apomorphine. The following week, the same animals were pretreated with 150.0 mg/kg of AMPT 135 min. prior to their retest with 10.0 mg/kg of apomorphine.

Apomorphine-Induced Rotation: Interaction with Unilateral Caudate Lesions

Twenty rats were tested initially (Day 1) with apomorphine (10.0 mg/kg). Based on the magnitude of rotation, the animals were matched for equivalent behavior and paired. Each member of a pair was assigned to one of two groups (N= 10/group). On Day 5, all rats received unilateral lesions of the caudate nucleus. In one group (IPSI), the lesions were made in the caudate nucleus ipsilateral to the initial direction of rotation, whereas, in the other group (CONTRA), the lesions were made in the caudate nucleus contralateral to the initial direction of rotation. On Day 8, all rats were tested again with the same dose (10.0 mg/kg) of apomorphine.

Surgery was performed under methohexital anesthesia, and lesions were made by a direct anodal current of 2 ma. for 15 seconds. Stereotaxic coordinates were 2.0 mm anterior to bregma, 3.0 mm lateral to the midline, and 5.5 mm from the dura (Pellegrino & Cushman, 1967). Following their use in the experiment, all animals were killed and perfused with 10% formalin. Their brains were removed and immersed in formalin for several days before sections (40 µm stained with Luxol blue and cresyl violet) were made and histological examination was conducted. All lesions were 2.5 - 3.0 mm in diameter and centrally located within the caudate nucleus.

NEUROCHEMICAL METHODS

Sixty-three rats were individually placed in a rotometer, and after 15 minutes 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 minutes 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 four 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 striati and the tel-diencephalon halves respectively. After centrifuging the samples at 27,000 x g for 20 minutes, 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 (1968, 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 & Taylor, 1972) and then striatal dopamine and tel-diencephalic norephinephrine were

determined by the spectrofluorometric method (Laverty & 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, Murdick, and Ray (1969), and Anggard, Gunne, and Niklasson (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 minutes. The tubes were then centrifuged at about 500 x g for 5 minutes, 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 minutes. 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) was performed according to the conditions in Table 1.

TABLE 1

CONDITIONS FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF AMPHETAMINE

Column	Temperatures (°C)			Carrier Gas Flow
	Inlet	Column	Detector	
4 feet, glass, 4 mm I.D., 6 mm O.D., 3% UC W-98 on 80-100 mesh Chromsorb W AW-DMCS high performance	250	175	275	Ar (5%) - Me (95%) 55 ml/min.

BEHAVIORAL RESULTS

Dose-Response Relationships

For all dose-response relationships the mean number of net positive rotations (i.e. dominant minus opposite) and standard error of the mean for each group of six rats is plotted as a function of dose. Analysis of variance and two-tailed t-tests were used for data analysis.

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 $p < 0.001$) 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. Figure 2 illustrates the time course of amphetamine-induced rotation. The 1.25 mg/kg dose has its peak effect between 25 and 45 minutes, whereas the 20.0 mg/kg dose induces the greatest rotation within the first 5 minutes after injection. The 20.0 mg/kg dose produced more variability from animal to animal and more frequent rotations to the opposite direction.

Amphetamine and AMPT dose-response interactions. Table 2 lists the net rotations induced by amphetamine alone and those elicited by amphetamine after pretreating rats with AMPT. The

Figure 1. Dose-response relationship for d-amphetamine-induced rotation (mean $\pm$ standard error). Rats were placed in the rotometer 15 minutes prior to the injection of d-amphetamine. Rotations were then recorded for 60 minutes.

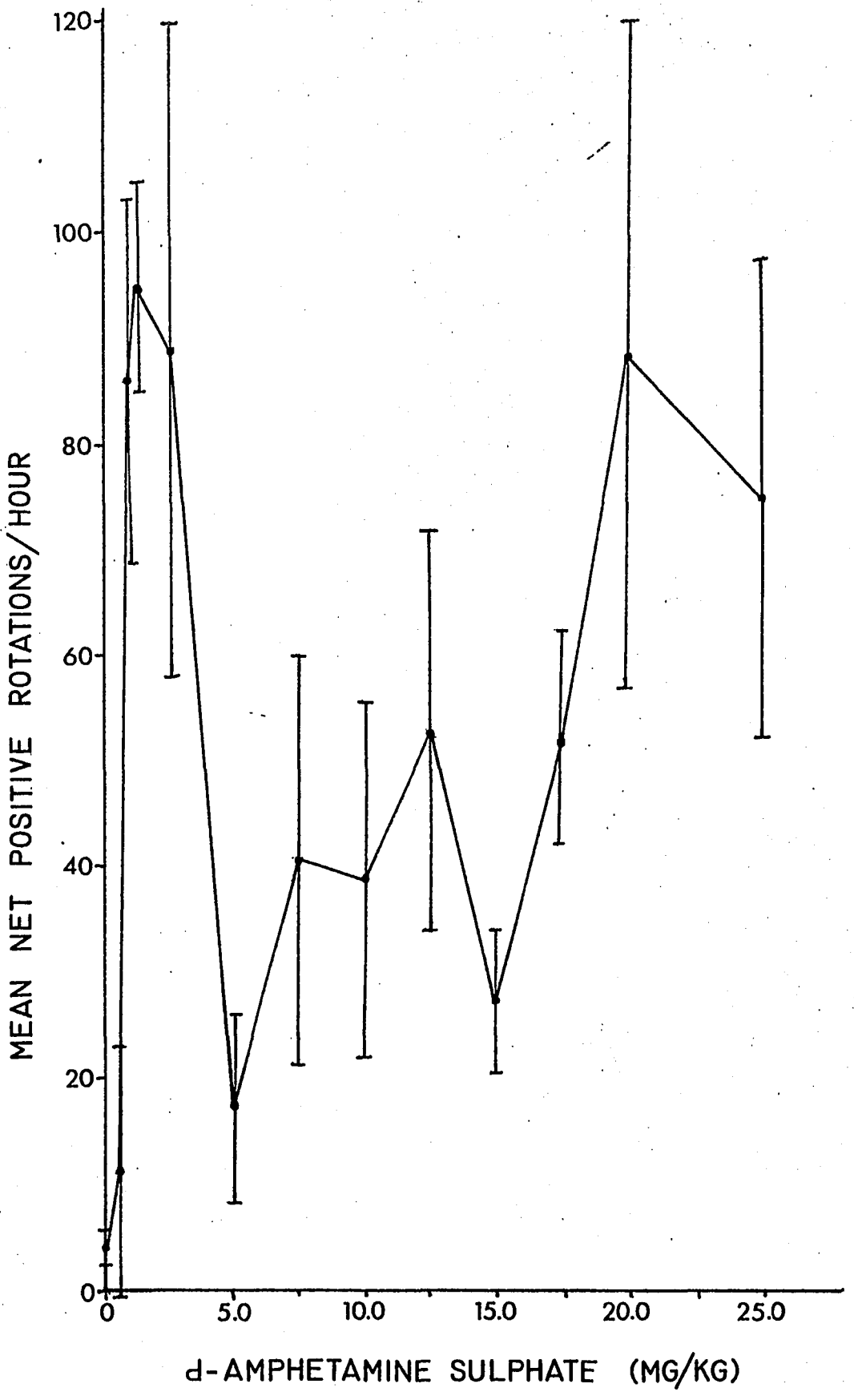

120
100
80
60
40
20
0
MEAN NET POSITIVE ROTATIONS/HOUR
0
5.0
10.0
15.0
20.0
25.0
d-AMPHETAMINE SULPHATE (MG/KG)

Figure 2. Time course of rotation (mean ± standard error) preceding and following injection (arrow) of 1.25 mg/kg (upper figure) and 20.0 mg/kg (lower figure) of d-amphetamine.

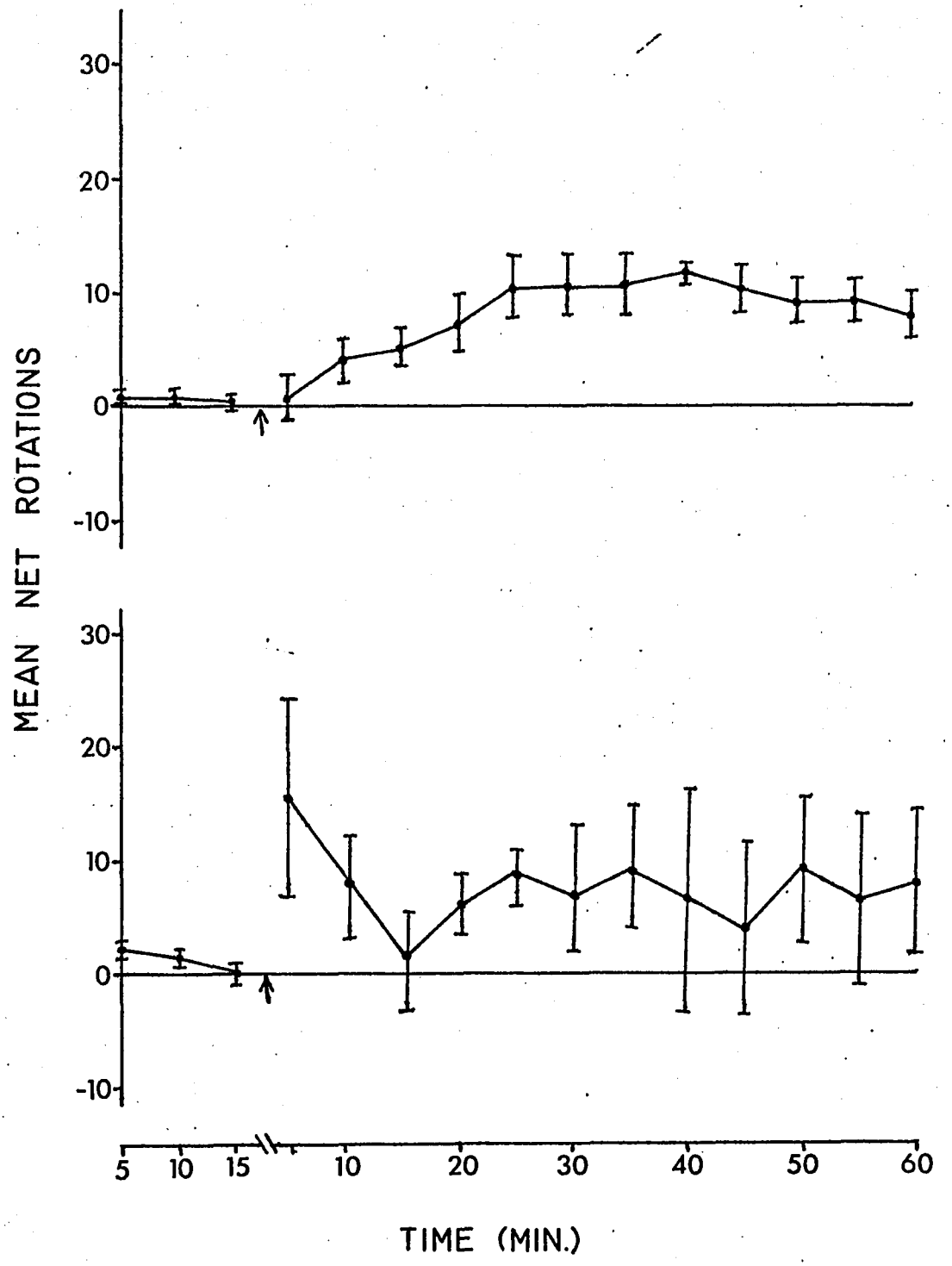

30
20
10
0
-10
MEAN NET ROTATIONS
30
20
10
0
-10
5
10
15
10
20
30
40
50
60
TIME (MIN.)

TABLE 2

AMPHETAMINE-AMPT DOSE-RESPONSE INTERACTIONS

Mean Net Positive Rotations (± Standard Error)

Dose	Amphetamine	Amphetamine-AMPT
0.0	3.8 ± 1.7	1.8 ± 0.9
1.0*	85.0 ± 19.9	10.7 ± 3.5
2.5**	88.7 ± 31.9	12.0 ± 4.9
5.0	17.2 ± 9.0	20.3 ± 5.9
15.0	27.2 ± 7.1	52.3 ± 16.0
25.0	74.7 ± 22.5	51.7 ± 18.8

* $p < 0.005$ (t-tests)
** $p < 0.05$

For the Amphetamine-AMPT interaction, rats were pretreated with AMPT (150.0 mg/kg) 135 minutes prior to the injection of amphetamine.

main effects of a two-way analysis of variance indicated that there was a significant ($p < 0.01$) effect of dose but no significant ($p > 0.1$) treatment (i.e. amphetamine vs. amphetamine and AMPT) effect. However, the significant ($p < 0.05$) dose x treatment interaction necessitated further analysis between treatments using multiple t-tests. In Table 2, rotations of AMPT treated rats were significantly less than the rotations elicited by amphetamine alone at the doses of 1.0 and 2.5 mg/kg ($p < 0.01$ and $p < 0.05$ respectively). At higher doses of amphetamine (i.e. greater than 5.0 mg.kg), rotations were not significantly ($p > 0.1$) reduced by pretreating the animals with AMPT. In Figure 3 the time course of rotation induced by high doses of amphetamine (i.e. 25.0 mg/kg) alone differs from that elicited by the same dose after pretreatment with AMPT. It appears that AMPT prevented the rapid onset of rotation immediately following the injection of amphetamine and shifted the period of peak rotation to the earlier portion of the test session.

Amphetamine and haloperidol dose-response interactions. A two-way analysis of variance indicated that the only significant ($p < 0.01$) main effect, in Table 3, was due to dose. The t-tests performed as a consequence of the significant ($p < 0.01$) dose x treatment interaction indicated that mean rotations were significantly ($p < 0.05$) reduced, at the doses of 1.0 and 2.5 mg/kg of amphetamine, in animals pretreated with haloperidol. At the higher doses of amphetamine (i.e. greater than 5.0 mg/kg), rotations were not significantly ($p > 0.1$) reduced by treating the rats with haloperidol. However, as with AMPT, the time

Figure 3. Time course of rotation preceding and following injection (arrow) of 25.0 mg/kg of d-amphetamine (●——●) in rats pretreated with either AMPT (●---●, 150.0 mg/kg) or haloperidol (x---x, 1.0 mg/kg).

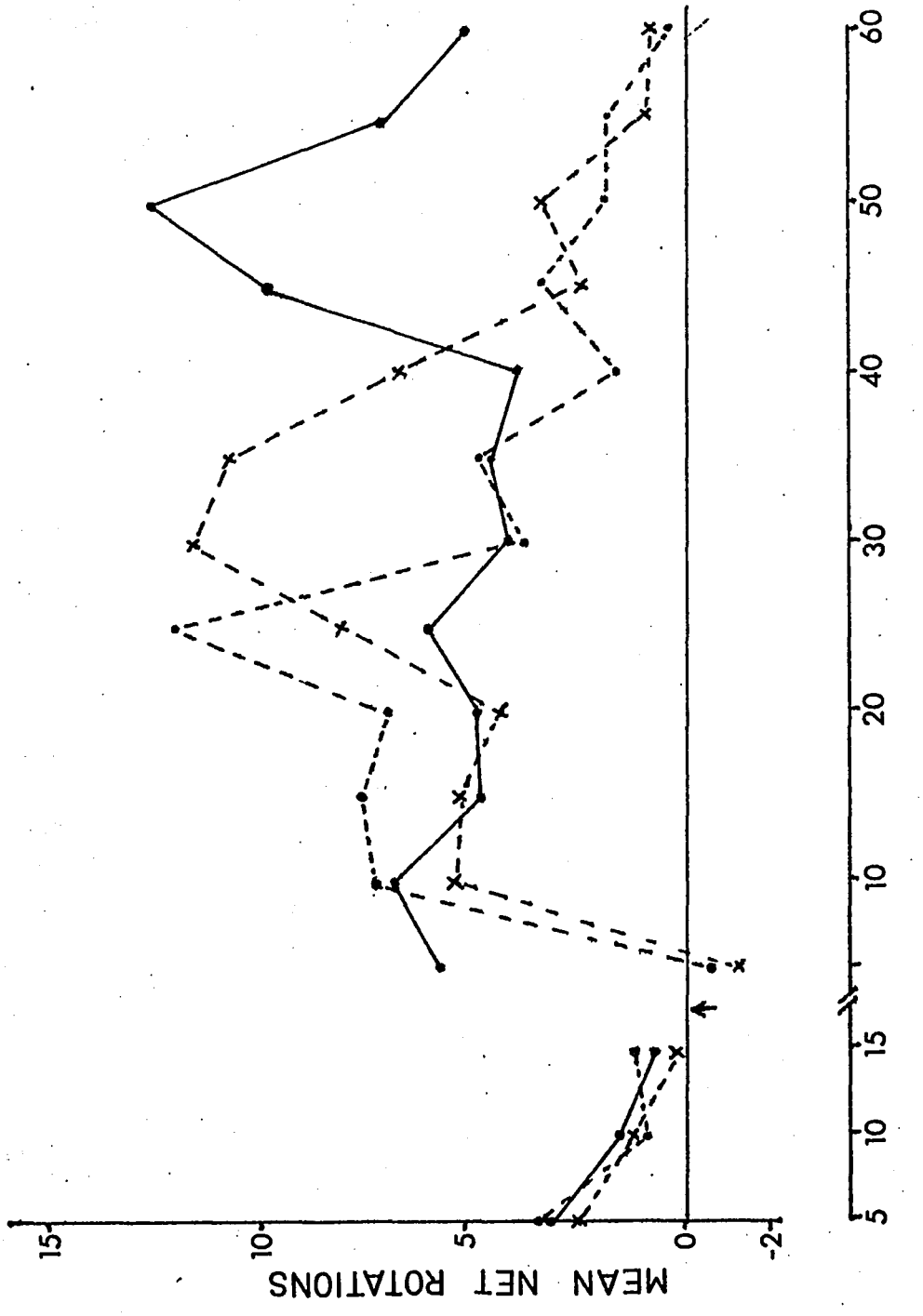

MEAN NET ROTATIONS
15
10
5
0
-2
5
10
15
10
20
30
40
50
60
TIME (MIN.)

TABLE 3

AMPHETAMINE-HALOPERIDOL DOSE-RESPONSE INTERACTIONS

Mean Net Positive Rotations (± Standard Error)

Dose	Amphetamine	Amphetamine-Haloperidol
0.0	3.8 ± 1.7	0.8 ± 0.5
1.0*	85.0 ± 19.9	0.8 ± 0.3
2.5**	88.7 ± 31.9	0.8 ± 0.3
5.0	17.2 ± 9.0	3.7 ± 1.1
15.0	27.2 ± 7.1	34.2 ± 11.3
25.0	74.7 ± 22.5	57.2 ± 11.4

* $p < 0.005$ (t-tests)
** $p < 0.05$

For the Amphetamine-Haloperidol interaction, rats were pretreated with haloperidol (1.0 mg/kg) 60 minutes prior to the injection of amphetamine.

course of rotation induced by the 25.0 mg/kg dose of amphetamine was markedly altered by the prior administration of haloperidol (Figure 3). Pretreating the rats with haloperidol prevented the rapid onset of rotation immediately following the administration of amphetamine and shifted the period of peak rotation to the earlier portion of the test session. Rotations to the opposite direction, characteristic of the high doses of amphetamine, were considerably reduced by haloperidol or AMPT pretreatment.

Amphetamine and DDC dose-response interactions. A two-way analysis of variance performed on the data in Table 4 indicated that although there was a significant ($p < 0.01$) main effect due to dose, there was neither a significant ($p > 0.1$) treatment (i.e. amphetamine vs. amphetamine and DDC) effect nor a significant ($p > 0.1$) dose x treatment interaction. Thus DDC had no significant effect on amphetamine-induced rotation..

Apomorphine dose-response interactions

In Figure 4, the dose-response relationship for apomorphine-induced rotation, all doses equal to or greater than 2.5 mg/kg elicited significant ($p < 0.05$) rotation. The curve appears to plateau at 10.0 mg/kg since there is no significant ($p > 0.1$) difference between the effects of 10.0 and 50.0 mg/kg. The rotation induced by 10.0 mg/kg of apomorphine, in Figure 5, reaches its maximum during the first 5 minutes after injection, and the direction of rotation remains constant throughout the entire hour.

L-DOPA dose-response

In Figure 6, only the dose of 300.0 mg/kg of L-DOPA induced significant ($p < 0.05$) rotation in rats pretreated with MK 486. The inhibitor alone did not cause any significant

TABLE 4

AMPHETAMINE-DDC DOSE-RESPONSE INTERACTIONS

Dose	Mean Net Positive Rotations (± Standard Error)	
	Amphetamine	Amphetamine-DDC
0.0	3.8 ± 1.7	13.0 ± 4.5
1.0	85.0 ± 19.9	156.0 ± 68.4
2.5	88.7 ± 31.9	61.7 ± 24.1
5.0	17.2 ± 9.0	33.3 ± 11.8
15.0	27.2 ± 7.1	121.0 ± 26.3
25.0	74.7 ± 22.5	87.0 ± 21.6

For the Amphetamine-DDC interaction, rats were pretreated with DDC (200.0 mg/kg) 45 minutes prior to the injection of amphetamine.

Figure 4. Dose-response relationship for apomorphine-induced rotation (mean ± standard error). Rats were placed in the rotometer 15 minutes prior to the injection of apomorphine. Rotations were then recorded for 60 minutes.

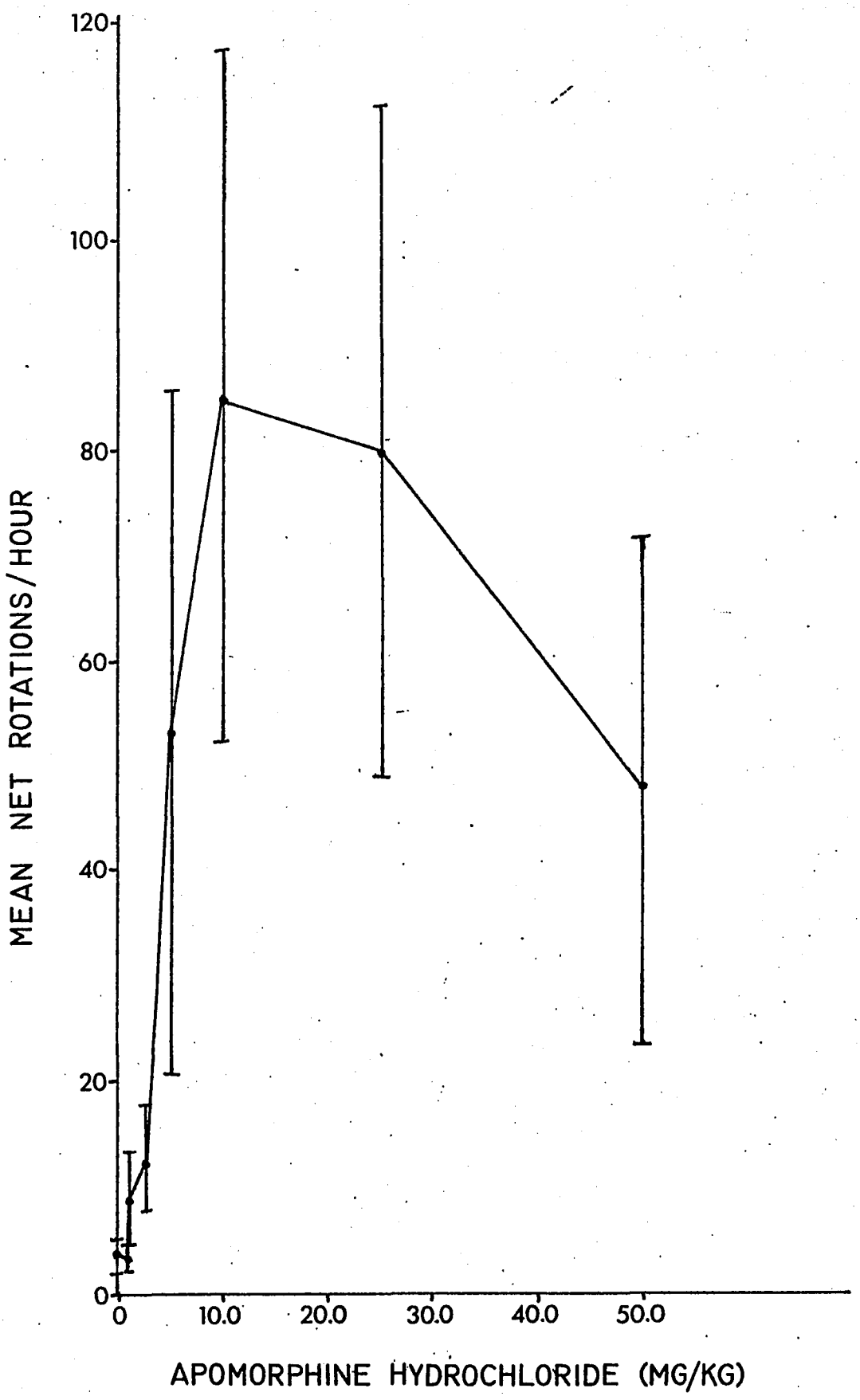

120
100
80
60
40
20
0
MEAN NET ROTATIONS/HOUR
0
10.0
20.0
30.0
40.0
50.0
APOMORPHINE HYDROCHLORIDE (MG/KG)

Figure 5. Time course of rotation (mean ± standard error) preceding and following injection (arrow) of 10.0 mg/kg of apomorphine.

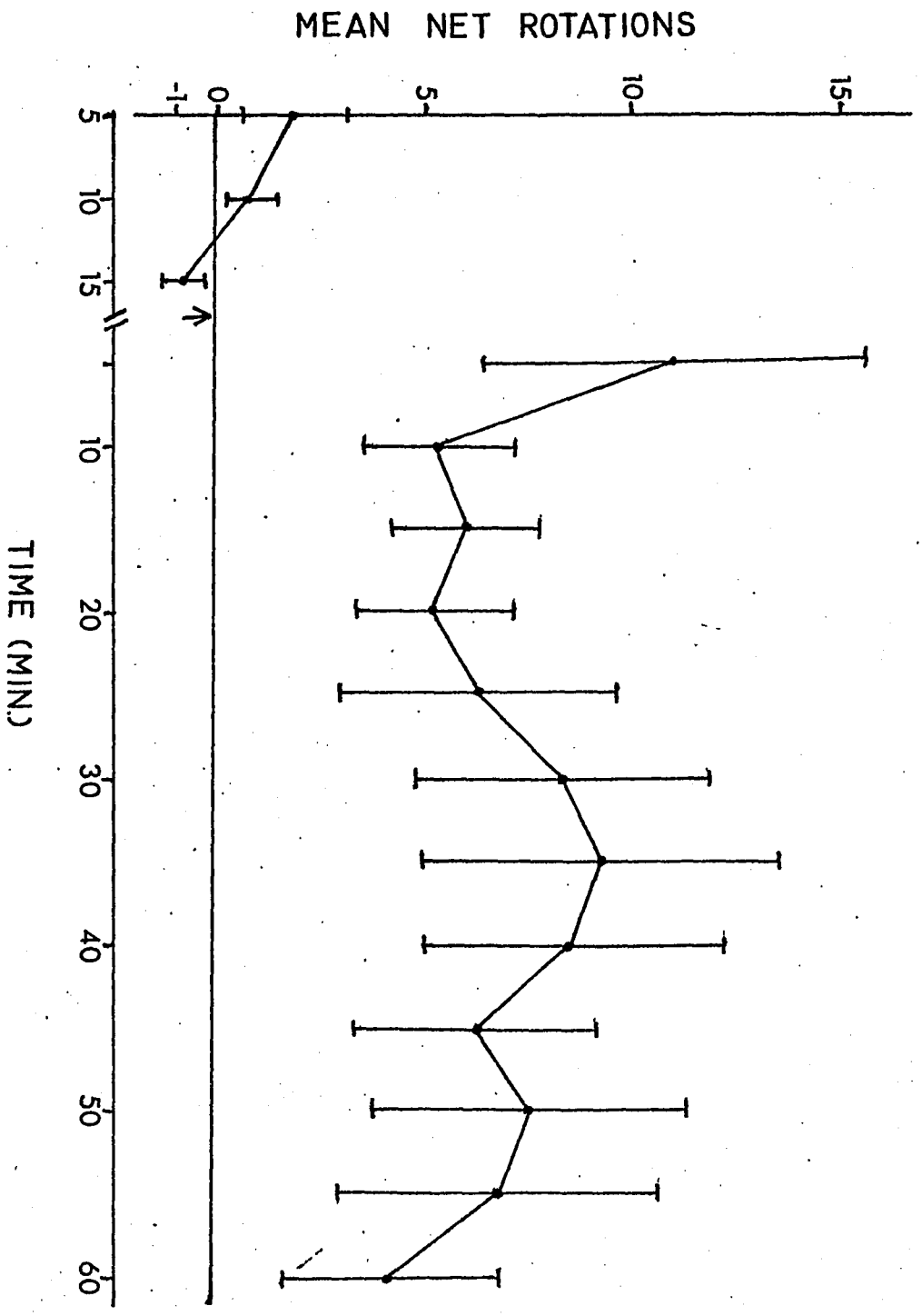
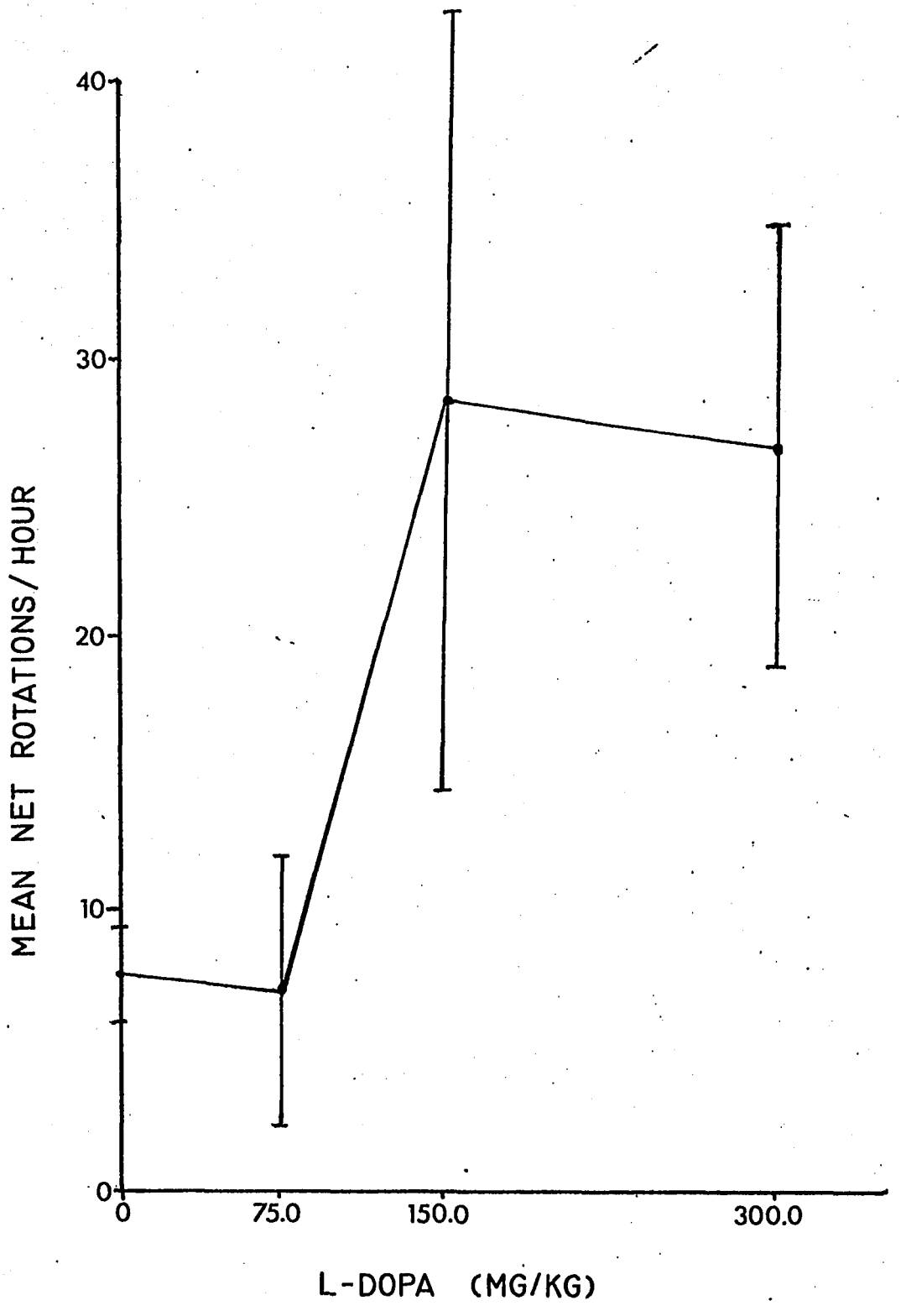

MEAN NET ROTATIONS
-1
0
5
10
15
TIME (MIN.)
5
10
15
10
20
30
40
50
60

Figure 6. Dose-response relationship for L-DOPA-induced rotation (mean ± standard error). Fifteen minutes after the administration of MK 486 (150.0 mg/kg), rats were placed in the rotometer 15 minutes prior to the injection of L-DOPA. Rotations were then recorded for 60 minutes.


40
30
20
10
0
MEAN NET ROTATIONS/HOUR
0
75.0
150.0
300.0
L-DOPA (MG/KG)

($p > 0.1$) rotation as compared to the saline controls of Figure 1. The time course of the rotation induced by L-DOPA (300.0 mg/kg), in Figure 7, is marked by periods of frequent rotations to the opposite direction, and during any 5 minute period mean rotations never exceed five.

Haloperidol dose-response

Haloperidol-induced rotation, illustrated in Figure 8, is characterized by a steep dose-response relationship. Only the dose of 0.125 mg/kg elicited significant ($p < 0.05$) rotation. Rotations, at all other doses, are not significantly ($p > 0.1$) different from those of the control group. The time course of haloperidol-induced rotation in Figure 9, indicates that although the direction of rotation is reasonably constant, the magnitude of rotation is rather minimal. Very few rotations are elicited during any 5 minute period.

Scopolamine dose-response

At all doses of scopolamine in Figure 10, 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) difference in effect over a dose range of greater than two log units. Figure 11, the time course of scopolamine-induced rotation, shows that the drug does not elicit marked rotation during any of the 5 minute periods.

Pilocarpine dose-response

Pilocarpine did not induce any significant rotational

Figure 7. Time course of rotation (mean ± standard error) preceding and following the injection (arrow) of 300.0 mg/kg of L-DOPA in rats pretreated with MK 486.

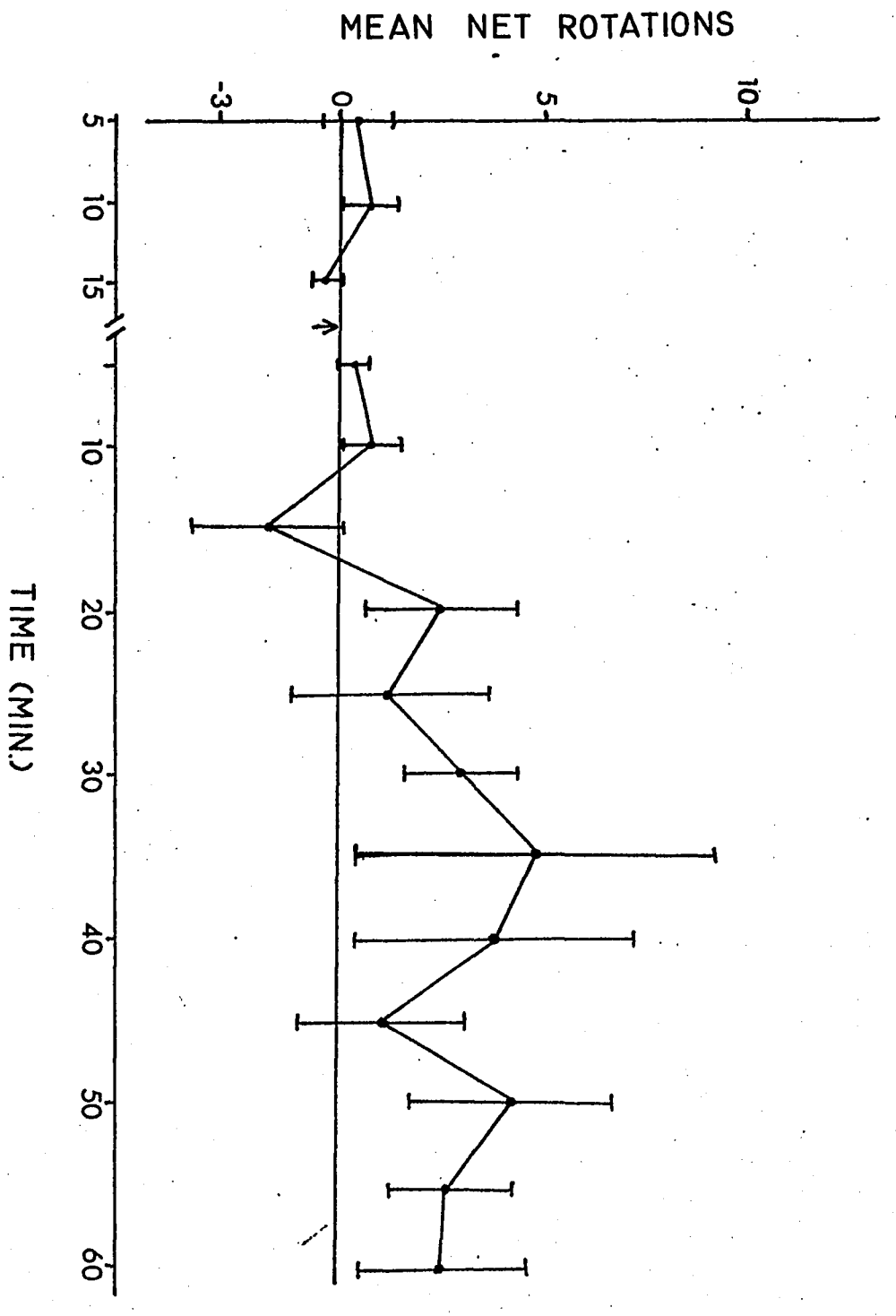

MEAN NET ROTATIONS
-3
0
5
10
TIME (MIN.)
5
10
15
10
20
30
40
50
60

Figure 8. Dose-response relationship for haloperidol-induced rotation (mean ± standard error). Forty-five minutes after the administration of haloperidol, rats were placed in the rotometer. Fifteen minutes later, rotations were recorded for 60 minutes.

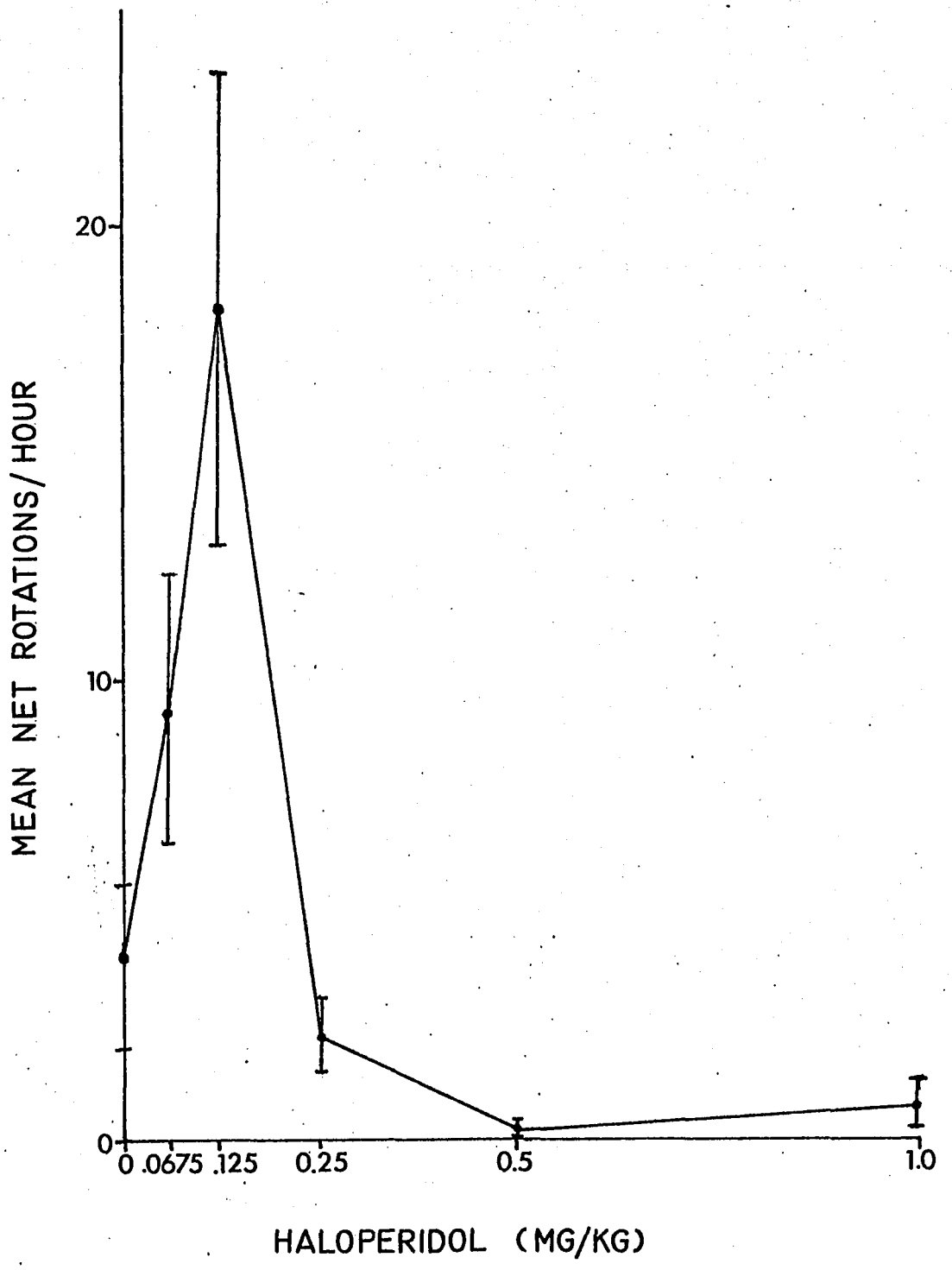

MEAN NET ROTATIONS/HOUR
20
10
0
0 .0675 .125 0.25 0.5 1.0
HALOPERIDOL (MG/KG)

Figure 9. Time course of rotation (mean ± standard error) 45 minutes following the injection of 0.125 mg/kg of haloperidol.

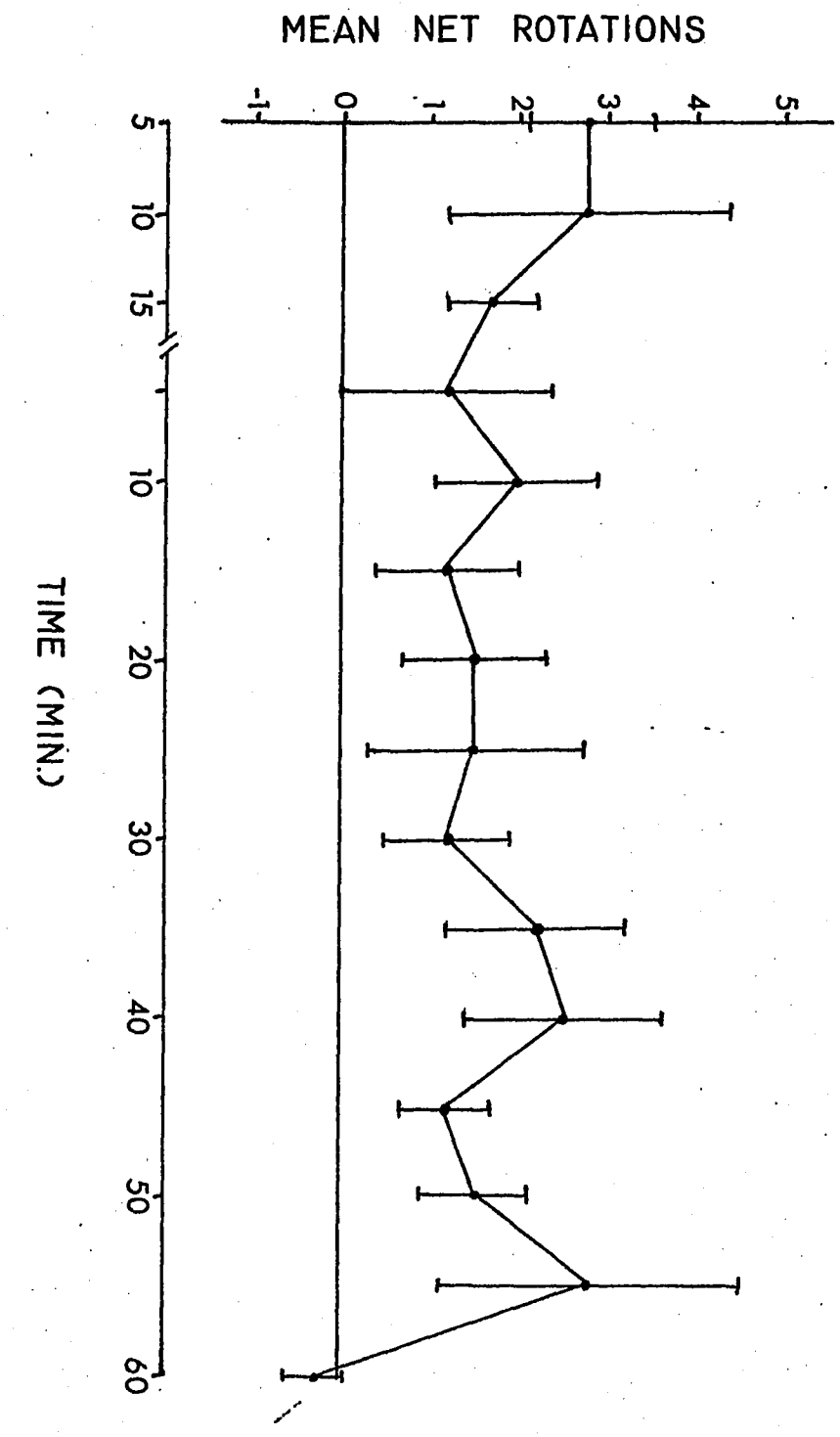

MEAN NET ROTATIONS
-1
0
1
2
3
4
5
5
10
15
10
20
30
40
50
60
TIME (MIN.)

Figure 10. Dose-response relationship for scopolamine-induced rotation (mean ± standard error). Rats were placed in the rotometer 15 minutes prior to the injection of scopolamine. Rotations were then recorded for 60 minutes.

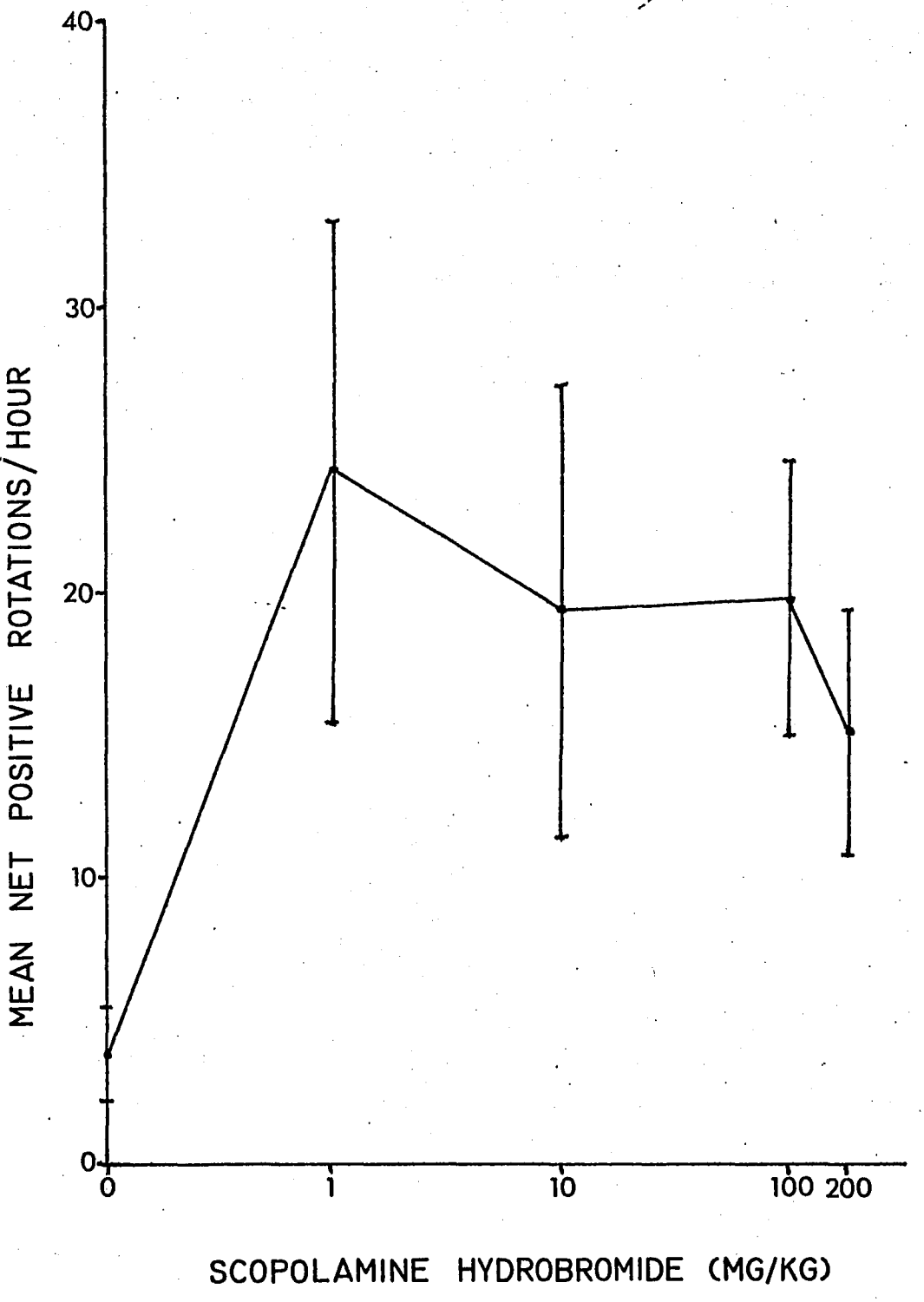
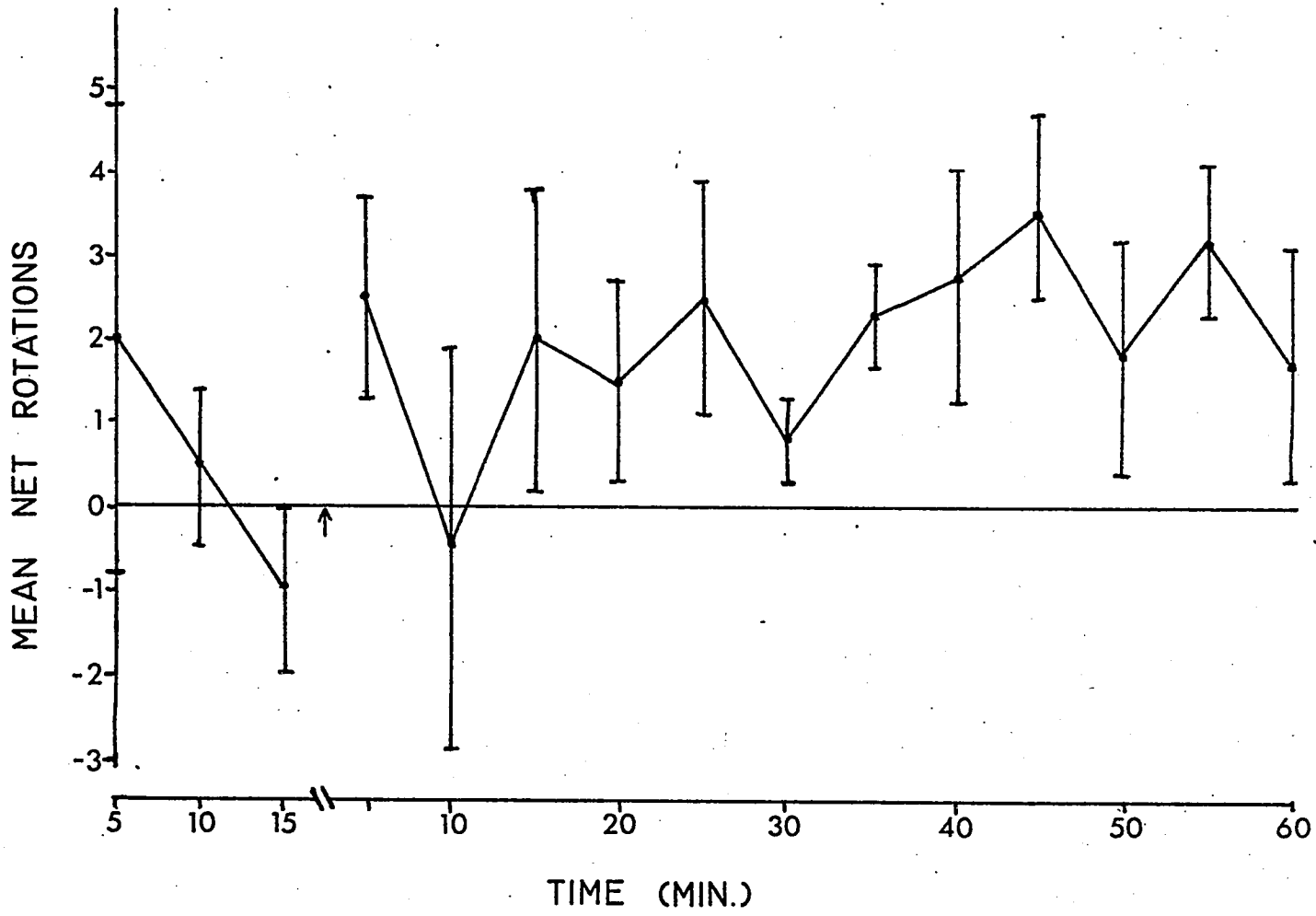

40
30
20
10
0
MEAN NET POSITIVE ROTATIONS/HOUR
0
1
10
100 200
SCOPOLAMINE HYDROBROMIDE (MG/KG)

Figure 11. Time course of rotation (mean ± standard error) preceding and following injection (arrow) of 1.0 mg/kg of scopolamine.


MEAN NET ROTATIONS
5
4
3
2
1
0
-1
-2
-3
5
10
15
10
20
30
40
50
60
TIME (MIN.)

behavior. At all doses tested, mean net rotations were less than five during the test hour.

Consistency of Pharmacologically Induced Rotation: Directional and Quantitative Correlations

Net rotations (i.e. rotations to the left minus rotations to the right) were determined for Day 1 and Day 8 for each rat. These data were then analyzed, for their consistency from week to week, by Chi Square (where applicable), paired *t*-tests, and linear regression analysis. As an indication of the consistency of each rat's directional preference independent of variations in the total number of rotations, "% Dominance" (i.e. the percent of total rotations in the dominant direction) was included in the Tables.

Amphetamine

As shown in Table 5, the direction of amphetamine-induced rotation for each rat is unchanged from week to week. Some rats consistently rotate to the left (indicated by the minus sign), whereas others consistently rotate to the right (plus sign). Not only is the direction of rotation consistent, but the magnitude of rotation is significantly correlated ($p < 0.05$) and not significantly different ($p > 0.1$, paired *t*-tests) from week to week.

Apomorphine

As with amphetamine-induced rotation, the rotation elicited by apomorphine, in Table 6, also is consistent from week to week. Rats predominantly rotate either to the left or to the right, but do not change this preference. Again, the rotational

TABLE 5

CONSISTENCY OF AMPHETAMINE-INDUCED ROTATION

Rat No.	Net Rotations (% Dominance) for 1.0 mg/kg Day 1	Day 8
1	+ 113 (97.5)	+ 136 (99.3)
2	- 113 (93.8)	- 80 (100.0)
3	- 67 (100.0)	- 133 (100.0)
4	- 53 (94.9)	- 50 (90.3)
5	+ 147 (85.5)	+ 66 (83.7)
6	+ 15 (80.0)	+ 18 (84.6)
7	+ 12 (57.7)	+ 36 (97.4)
8	+ 225 (98.3)	+ 86 (98.9)
9	- 75 (91.2)	- 46 (87.1)
10	+ 149 (96.9)	+ 216 (99.1)
11	+ 85 (74.0)	+ 128 (88.6)
12	- 48 (98.0)	- 105 (99.1)
13	- 76 (93.2)	- 134 (96.5)
14	+ 44 (72.0)	+ 11 (82.4)
15	- 158 (97.0)	- 164 (96.1)
Mean	92.0 (88.7)	93.9 (93.5)

For Day 1 versus Day 8, the magnitude of net rotation is significantly correlated ($r = 0.51$, $p < 0.05$, linear regression analysis) and the means are not significantly different ($p > 0.1$, paired t-tests).

TABLE 6

CONSISTENCY OF APOMORPHINE-INDUCED ROTATION

Rat No.	Net Rotations (% Dominance) for 10.0 mg/kg Day 1	Day 8
1	+ 226 (100.0)	+ 276 (100.0)
2	- 49 (94.5)	- 29 (91.4)
3	- 26 (72.4)	- 126 (82.5)
4	- 62 (91.9)	- 60 (91.7)
5	- 70 (91.7)	- 49 (96.2)
6	+ 42 (61.3)	+ 62 (62.8)
7	+ 123 (100.0)	+ 126 (100.0)
8	- 71 (98.6)	- 56 (100.0)
9	- 200 (87.3)	- 215 (85.5)
10	- 12 (70.0)	- 33 (97.1)
11	+ 12 (63.6)	+ 24 (83.3)
12	+ 40 (91.7)	+ 99 (94.6)
13	+ 24 (77.3)	+ 33 (88.4)
14	+ 83 (97.7)	+ 23 (64.2)
15	- 24 (76.1)	- 10 (77.8)
16	- 171 (95.2)	- 169 (94.2)
17	+ 39 (91.5)	+ 83 (84.9)
18	+ 111 (98.3)	+ 83 (100.0)
19	- 124 (98.4)	- 226 (99.1)
Mean	79.4 (87.2)	93.8 (89.1)

For Day 1 versus Day 8, the magnitude of net rotation is significantly correlated ($r = 0.84$, $p < 0.001$, linear regression analysis) and the means are not significantly different ($p > 0.1$, paired *t*-tests).

magnitudes are significantly correlated ($p < 0.001$) and not significantly ($p > 0.1$, paired *t*-tests) changed from Day 1 to Day 8.

Scopolamine

For the group as a whole, in Table 7, the direction of rotation is significantly consistent ($p < 0.01$, Chi Square) from week to week. Only one of the rats tested with scopolamine rotated to the opposite direction on Day 8. In contrast to amphetamine- or apomorphine-induced rotation, the magnitude of rotation elicited by the weekly administration of scopolamine is not significantly correlated ($p > 0.1$) and is significantly greater on Day 8 than on Day 1 ($p < 0.001$, paired *t*-tests).

Directional Consistency between Drugs which Induce Rotation

The rotation induced on Day 1 and Day 8, by the various drugs, was compared for consistency of direction by Chi Square, and where appropriate the significance of the magnitude of rotation was determined by paired *t*-tests and linear regression analysis.

Amphetamine (low dose vs. high dose)

As shown in Table 8, the rotation induced by the 1.0 and 20.0 mg/kg doses of amphetamine is not necessarily in the same direction for both doses ($p > 0.1$, Chi Square). Some rats rotate in one direction with the low dose of amphetamine and then turn to the opposite direction with the high dose. Rats tested with 20.0 mg/kg rotated in the same direction as Day 1, during the first few minutes of the test session. However, during the later portions of the same test session, a prepon-

TABLE 7

CONSISTENCY OF SCOPOLAMINE-INDUCED ROTATION

Rat No.	Net Rotations (% Dominance) for 1.0 mg/kg Day 1	Day 8
1	+ 28 (58.9)	+ 76 (70.9)
2	- 48 (72.6)	- 75 (91.2)
3	- 60 (75.9)	- 71 (91.1)
4	+ 25 (59.4)	- 21 (41.0)
5	+ 20 (63.9)	+ 97 (82.1)
6	+ 21 (67.2)	+ 105 (99.1)
7	+ 16 (56.0)	+ 151 (93.1)
8	+ 25 (72.7)	+ 80 (93.5)
9	+ 28 (81.8)	+ 86 (93.0)
10	- 39 (62.3)	- 99 (84.1)
11	- 40 (70.0)	- 63 (90.9)
12	+ 69 (96.0)	+ 149 (84.5)
13	- 85 (92.9)	- 118 (84.3)
|Mean|	38.8 (71.5)	91.8 (84.5)

For Day 1 versus Day 8, the direction of net rotation is not significantly changed ($p < 0.01$, Chi Square), the magnitude is not significantly correlated ($r = 0.23$, $p > 0.1$, linear regression analysis), and the means are significantly different ($p < 0.001$, paired *t*-tests).

TABLE 8

CONSISTENCY BETWEEN LOW AND HIGH DOSE OF AMPHETAMINE-INDUCED ROTATION

	Net Rotations	
Rat No.	Day 1 Amphetamine (1.0 mg/kg)	Day 8 Amphetamine (20.0 mg/kg)
1	+ 48	+ 17
2	+ 149	- 45
3	- 13	- 80
4	- 166	- 25
5	- 26	- 43
6	+ 69	- 23
7	+ 105	+ 121
8	+ 33	+ 17
9	+ 19	- 37
|Mean|	69.8	45.3

For Day 1 versus Day 8, the direction of net rotation is not necessarily the same ($p > 0.1$, Chi Square), the magnitude is not significantly correlated ($r = 0.07$, $p > 0.1$, linear regression analysis), and the means are not significantly different ($p > 0.1$ paired t-tests).

derance of rotations to the opposite direction were recorded. Not only is the direction of rotation not consistent, but in contrast to the data presented in Table 5, the magnitude of rotation, for the whole hour, is not significantly correlated ($p > 0.1$) between the low and high doses of amphetamine. Paired t-tests indicate that the number of net rotations induced by 1.0 or 20.0 mg/kg of amphetamine do not differ significantly ($p > 0.1$).

Amphetamine vs. apomorphine

The non-significant value ($p > 0.1$) for Chi Square, in Table 9, indicates that amphetamine and apomorphine do not necessarily induce rotation to the same direction for each rat. Half of the animals rotated in the same direction as that induced by apomorphine, whereas the remainder turned in the opposite direction after the administration of amphetamine on Day 8.

Amphetamine vs. scopolamine

As evident from Table 10, all rats rotated to the same direction on Day 1 and Day 8. Thus amphetamine and scopolamine induce rotation to the same direction.

Pharmacological Interactions: Effects on Rotational Behavior

The significance of the magnitude of rotation, for Day 1 vs. Day 8, was determined by paired t-tests and, where appropriate, linear regression analysis.

Amphetamine and AMPT interactions

TABLE 9

CONSISTENCY BETWEEN APOMORPHINE- AND AMPHETAMINE-INDUCED ROTATION

Net Rotations

Rat No.	Day 1 Apomorphine (10.0 mg/kg)	Day 8 Amphetamine (1.0 mg/kg)
1	- 28	- 47
2	- 17	- 10
3	+ 19	+ 51
4	+ 103	+ 12
5	- 25	+ 25
6	+ 79	+ 13
7	- 123	+ 14
8	+ 33	- 18
9	+ 134	- 10
10	+ 10	- 27
11	- 26	- 43
12	- 44	- 61
13	+ 69	- 74
14	- 133	+ 35

For Day 1 versus Day 8, the direction of net rotation is not necessarily the same ($p > 0.1$, Chi Square).

TABLE 10

CONSISTENCY BETWEEN SCOPOLAMINE- AND AMPHETAMINE-INDUCED ROTATION

Rat No.	Net Rotations: Day 1 Scopolamine (1.0 mg/kg)	Net Rotations: Day 8 Amphetamine (1.0 mg/kg)
1	- 75	- 179
2	- 74	- 145
3	+ 97	+ 33
4	+ 105	+ 85
5	+ 80	+ 38
6	+ 86	+ 145
7	- 99	- 166

Table 11 indicates that AMPT significantly reduced ($p < 0.01$, paired t-tests) the rotational behavior elicited by 1.0 mg/kg of amphetamine. Amphetamine-induced rotation, in rats pretreated with AMPT (Day 8), does not significantly correlate ($p > 0.1$) with the rotation of the same rats tested with amphetamine alone (Day 1).

Amphetamine and scopolamine interactions

As shown in Table 12, rotation induced by the combination of amphetamine and scopolamine (Day 8) was greater by 25% than rotation elicited by amphetamine alone (Day 1). However, due to the large variability among the animals, this effect was not significant.

Amphetamine and pilocarpine interactions

When combined with amphetamine, pilocarpine (1.0 mg/kg) significantly reduced ($p < 0.01$, paired t-tests) the rotation induced by 1.0 mg/kg of amphetamine shown in Table 13.

Apomorphine and AMPT interactions

From Table 14 it can be seen that AMPT had no significant effect ($p > 0.1$, paired t-tests) on the rotation induced by 10.0 mg/kg of apomorphine. Similar to Table 6 (consistency of apomorphine-induced rotation), Table 14 shows that the magnitude of rotation is significantly correlated ($p < 0.05$) from Day 1 to Day 8 even after pretreatment with AMPT. The time course of apomorphine-induced rotation on Day 8 was not affected by AMPT and appeared similar to that shown in Figure 5.

Apomorphine-Induced Roation: Interaction with Unilateral Caudate Lesions

TABLE 11

AMPHETAMINE-AMPT INTERACTIONS: EFFECT ON AMPHETAMINE-INDUCED ROTATION

Net Rotations

Rat No.	Day 1 Amphetamine (1.0 mg/kg)	Day 8 Amphetamine (1.0 mg/kg)-AMPT (150.0 mg/kg)
1	+ 216	+ 71
2	+ 128	+ 22
3	- 105	- 37
4	- 134	- 23
5	+ 11	+ 2
6	- 164	+ 5
|Mean|	126.3	26.7

For Day 1 versus Day 8, the magnitude of net rotation is not significantly correlated ($r = 0.67$, $p > 0.1$, linear regression analysis) and the means are significantly different ($p < 0.01$, paired t-tests).

TABLE 12

AMPHETAMINE-SCOPOLAMINE INTERACTIONS: EFFECT ON AMPHETAMINE-INDUCED ROTATION

Net Rotations

Rat No.	Day 1 Amphetamine (1.0 mg/kg)	Day 8 Amphetamine (1.0 mg/kg) + Scopolamine (1.0 mg/kg)
1	- 179	- 258
2	- 145	- 159
3	+ 84	+ 15
4	+ 33	+ 77
5	+ 85	+ 158
6	+ 38	+ 113
7	+ 145	+ 193
8	- 166	- 126
|Mean|	109.4	137.4

For Day 1 versus Day 8, the mean net rotations are not significantly different ($p > 0.1$, paired t-tests).

TABLE 13

AMPHETAMINE-PILOCARPINE INTERACTIONS: EFFECT ON AMPHETAMINE-INDUCED ROTATION

Net Rotations

Rat No.	Day 1 Amphetamine (1.0 mg/kg)	Day 8 Amphetamine (1.0 mg/kg) + Pilocarpine (1.0 mg/kg)
1	+ 15	- 11
2	+ 31	+ 5
3	- 43	- 28
4	+ 69	+ 3
5	- 36	- 5
6	+ 46	+ 1
7	- 37	- 8
8	- 59	- 17
|Mean|	42.0	9.8

For Day 1 versus Day 8, the mean net rotations are significantly different ($p < 0.01$, paired t-tests).

TABLE 14

APOMORPHINE-AMPT INTERACTIONS: EFFECT ON APOMORPHINE-INDUCED ROTATION

	Net Rotations	
Rat No.	Day 1 Apomorphine (10.0 mg/kg)	Day 8 Apomorphine (10.0 mg/kg)-AMPT (150.0 mg/kg)
1	+ 99	+ 101
2	+ 33	+ 26
3	- 169	- 191
4	+ 83	+ 34
5	+ 83	+ 35
6	- 226	- 150
|Mean|	115.5	89.5

For Day 1 versus Day 8, the magnitude of net rotation is significantly correlated ($r = 0.86$, $p < 0.05$, linear regression analysis) and the means are not significantly different ($p > 0.1$, paired t-tests).

In Figure 12, a section through the center plane of a representative unilateral lesion of the caudate nucleus is shown. The lesion is about 3.0 mm in diameter and centrally located within the caudate nucleus. Following unilateral lesions of the caudate nucleus, all rats rotated to the side of the lesion after the administration of apomorphine on Day 8. However, the postoperative magnitude of rotation, in Table 15, was significantly ($p < 0.05$) greater in the IPSI group than in the CONTRA group.

Figure 12. Section through the center plane of a representative unilateral lesion of the caudate nucleus.

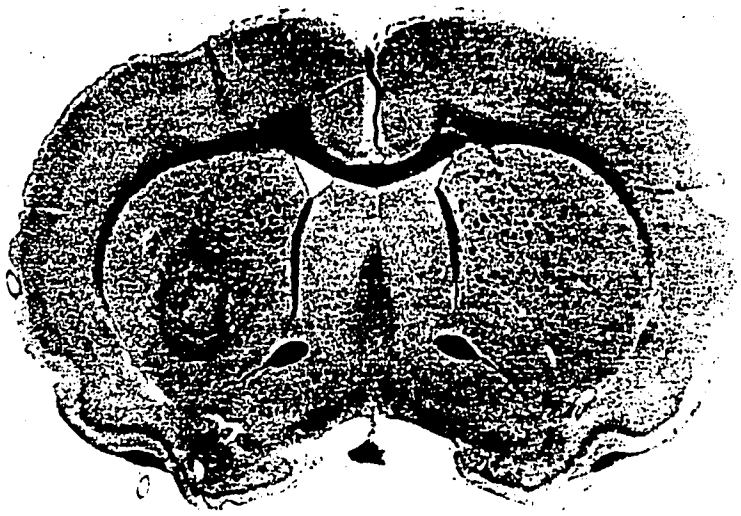


TABLE 15

MEAN NET POSITIVE ROTATIONS (+ STANDARD ERROR) INDUCED BY APOMORPHINE (10.0 mg/kg) BEFORE AND AFTER UNILATERAL LESIONS OF THE CAUDATE NUCLEUS

	Pre-operative	Post-operative
IPSI (N=10)	55.7 ± 14.8	242.7 ± 56.1
CONTRA (N=10)	59.2 ± 19.5	123.3 ± 39.9

Lesions were made either ipsilateral (IPSI) or contralateral (CONTRA) to the pre-operative direction of rotation.

Post-operative differences between IPSI and CONTRA groups were significant ($p < 0.05$, paired t tests).

NEUROCHEMICAL RESULTS

In Figure 13, the amphetamine content in the brain, after 30 minutes, is linearly related to the dose of amphetamine sulphate administered. Linear regression analysis shows that this relationship applied to both the striati ($r = 0.97$, $p < 0.001$) and tel-diencephalon ($r = 0.98$, $p < 0.001$) samples. On the other hand, Figure 14 indicates that striatal dopamine and tel-diencephalic norepinephrine levels, as a function of the injected dose of amphetamine, was non-monotonic. Norepinephrine was significantly 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.

It can be seen from Table 16 that there are no significant left-right differences for the neurochemical levels at each dose of amphetamine. Left-right differences for striatal and tel-diencephalic amphetamine also were not significant. When the drug and neurochemical levels were analyzed with respect

Figure 13. Mean (+ or - standard deviation) tel-diencephalic (●——●) and striatal (▲---▲) amphetamine levels following injection of d-amphetamine sulphate.

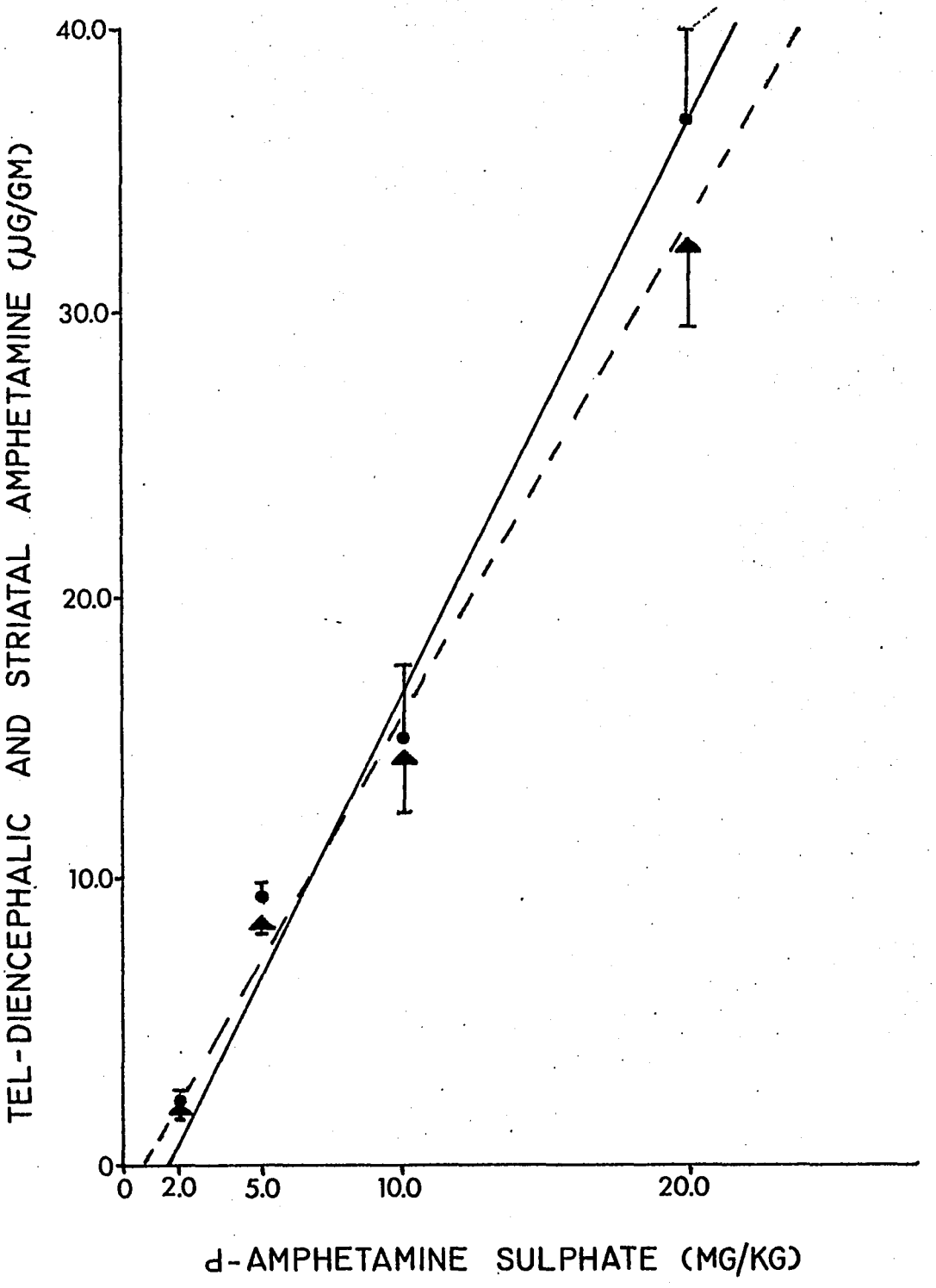

40.0
30.0
20.0
10.0
0
0
2.0
5.0
10.0
20.0
TEL-DIENCEPHALIC AND STRIATAL AMPHETAMINE (UG/GM)
d-AMPHETAMINE SULPHATE (MG/KG)

Figure 14. Mean (+ or - standard deviation) tel-diencephalic norepinephrine (▲---▲) and striatal dopamine (●——●) levels following injection of d-amphetamine sulphate.

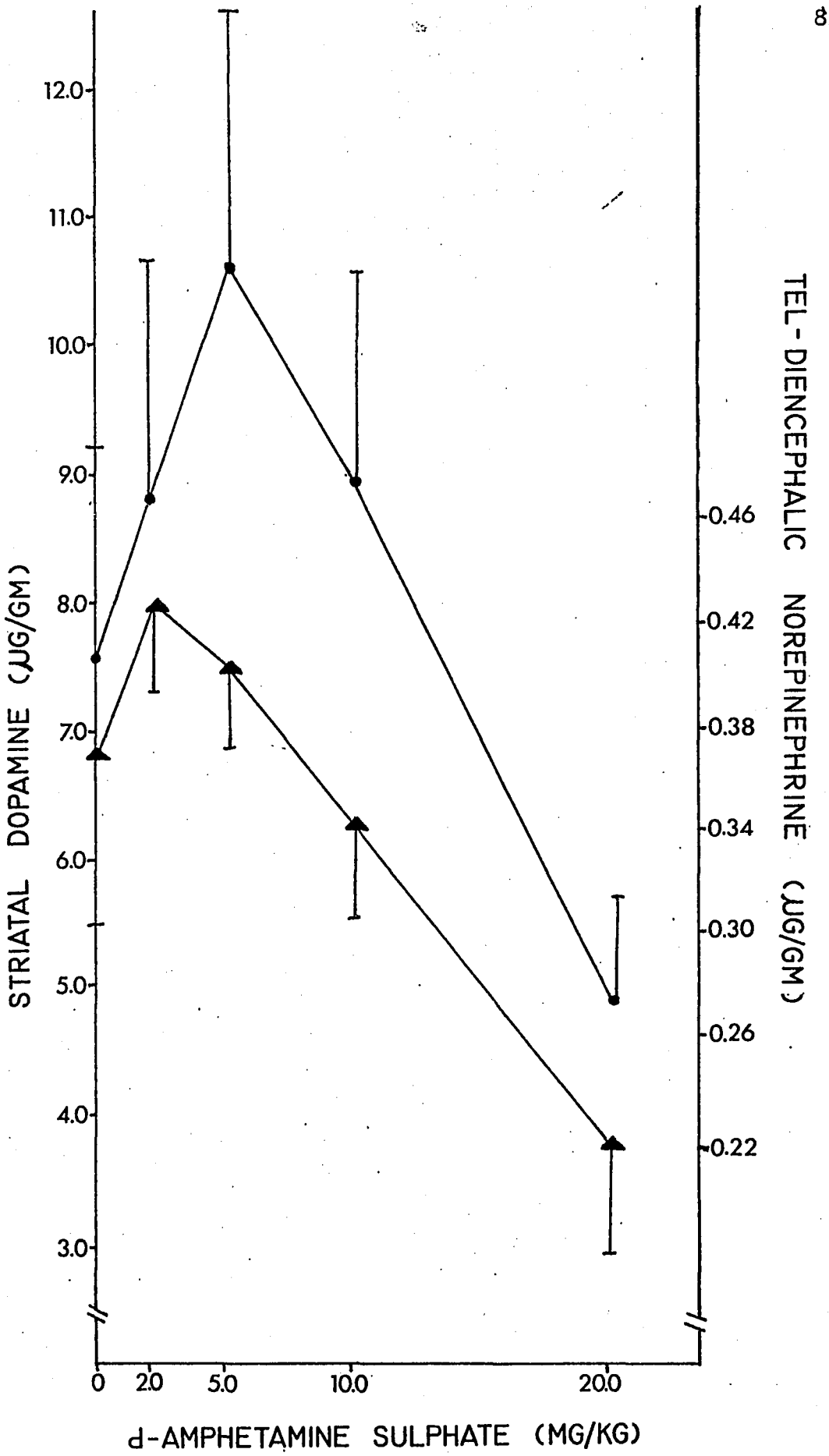

12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
STRIATAL DOPAMINE (µG/GM)
0.46
0.42
0.38
0.34
0.30
0.26
0.22
TEL-DIENCEPHALIC NOREPINEPHRINE (µG/GM)
0
2.0
5.0
10.0
20.0
d-AMPHETAMINE SULPHATE (MG/KG)

TABLE 16

MEAN (± STANDARD DEVIATION) UNILATERAL LEVELS (μg/g) OF AMPHETAMINE, TEL-DIENCEPHALIC NOREPINEPHRINE, AND STRIATAL DOPAMINE

Amphetamine sulphate (mg/kg)		Amphetamine		Norepinephrine	Dopamine
		Tel-diencephalon	Striatum	Tel-diencephalon	Striatum
0.0 (saline)	L	..	..	0.366 ± 0.070	7.639 ± 1.284
	R	..	..	0.378 ± 0.065	7.709 ± 1.756
	C	..	..	0.375 ± 0.064	7.481 ± 1.578
	I	..	..	0.368 ± 0.070	7.867 ± 1.443
2.0	L	2.25 ± 0.91	2.26 ± 0.96	0.435 ± 0.024	8.839 ± 2.194
	R	2.02 ± 0.90	2.24 ± 0.94	0.424 ± 0.043	8.797 ± 1.644
	C	2.11 ± 0.98	2.28 ± 0.96	0.438 ± 0.031	8.321 ± 1.746
	I	2.16 ± 0.84	2.23 ± 0.92	0.421 ± 0.037	*9.315 ± 1.984
5.0	L	8.59 ± 3.48	9.77 ± 3.99	0.401 ± 0.022	10.749 ± 1.888
	R	8.47 ± 2.90	9.01 ± 2.59	0.409 ± 0.037	10.379 ± 2.347
	C	8.63 ± 3.38	9.79 ± 3.48	0.408 ± 0.032	10.918 ± 2.329
	I	8.42 ± 3.02	8.99 ± 3.24	0.402 ± 0.029	10.210 ± 1.856
10.0	L	15.52 ± 3.26	13.84 ± 1.78	0.343 ± 0.035	8.879 ± 1.030
	R	14.52 ± 1.45	14.79 ± 2.20	0.344 ± 0.036	8.866 ± 1.185
	C	15.25 ± 2.57	15.10 ± 2.14	0.348 ± 0.036	9.154 ± 0.922
	I	14.79 ± 2.56	13.53 ± 1.59	0.339 ± 0.034	8.591 ± 1.198
20.0	L	37.74 ± 3.58	32.62 ± 3.19	0.195 ± 0.052	4.752 ± 0.688
	R	35.82 ± 2.34	31.86 ± 2.77	0.202 ± 0.039	4.996 ± 0.883
	C	37.39 ± 3.20	32.04 ± 2.74	0.196 ± 0.036	**5.403 ± 0.471
	I	36.17 ± 3.06	32.44 ± 3.26	0.201 ± 0.054	4.345 ± 0.672

L = left, R = right, C= contralateral, I = ipsilateral
*Differences significant at $p < 0.05$ (paired t tests)
**Differences significant at $p < 0.001$

to the direction of rotation, 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 $p < 0.001$ 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 (about 1%) do not display rotations sufficient enough (i.e. net rotations greater than or equal to ten per hour) to be distinguished from random movement. In spite of the large variability in rotation between animals, the direction (Tables 5, 6, & 7) and magnitude (Tables 5 & 6) of rotation are extremely stable for each individual rat.

Amphetamine-induced rotation

For amphetamine-induced rotation, the behavior elicited by the low doses appeared to be qualitatively different from the 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 occuring with lower doses in rats with unilateral caudate or SN lesions (Andén, 1970; Ungerstedt, 1971b; Ungerstedt & Arbuthnott, 1970). With all the drugs tested, the spherical

nature of the apparatus seemed to be an important factor in eliciting rotation. Previous investigators have not reported rotation in intact rats with low doses of amphetamine or other drugs possibly because rotation was observed either on a flat surface (Christie & Crow, 1971; Marsden & Guldberg, 1973; Naylor & Olley, 1972) or in a hemispherical rotometer (Ungerstedt, 1971b; Ungerstedt & Arbuthnott, 1970). On a flat surface, low doses of d-amphetamine appear, at first, only to cause the rat to become hyperactive. However, careful observation reveals that there are slight postural and locomotor asymmetries present, which are masked by the rat's hyperactivity and difficult to quantify. In a spherical rotometer, as that used in the present investigation, this hyperactivity is channeled into rotational behavior; it is probable that the enclosed apparatus also eliminated distracting environmental influences and induced the animal to move in an upright position. In addition, confinement in the rotometer could have produced a stressful situation which exacerbated the drug-induced rotation. This possibility will be discussed later, at greater length. The rat tested in a hemispherical rotometer with low doses of d-amphetamine is easily distracted and does not rotate, but rather climbs along the circular edge of the bowl-shaped apparatus. In contrast, with high doses of d-amphetamine, rotation was observed in any testing condition.

Amphetamine-induced rotation in rats with unilateral lesions of the NISTS, has been proposed to be due to the greater release of dopamine from the intact striatum which presumably results in a greater functional activity on that side as compared

to the lesioned side (Andén et al., 1966; Christie & Crow, 1971; Ungerstedt, 1971b; Ungerstedt & Arbuthnott, 1970). If this explanation is true, then amphetamine-induced rotation in normal rats could be explained by the same mechanism. Amphetamine could release more dopamine from one striatum as compared to the other, if one striatum intrinsically contained more dopamine or its content of the monoamine were more labile to the releasing action of the drug. Thus the biphasic dose-response relationship of amphetamine-induced rotation (Figure 1) could be explained by the effect of amphetamine's release of dopamine. Small doses of d-amphetamine would cause an unequal release of dopamine from either the right or left striatum. As the dose of amphetamine was increased, the right or left dopamine differences at the receptor would increase and rotation would be observed. Dopamine differences and concomitant rotation would be the greatest from about 1.0 to 2.5 mg/kg of amphetamine. With further increases in dose, the side which initially contained the greater amount or the more labile pool of dopamine would be close to depletion and the postsynaptic dopamine content would be nearly equal in the two striati. Thus at these doses of amphetamine (5.0 - 15.0 mg/kg), rotation would be expected to be at a minimum. High doses of amphetamine cause massive dopamine depletion (Brodie, Cho, & Gessa, 1970; Leonard & Shallice, 1971) in both striati (Glick, Jerussi, Waters, & Green, 1974). However, the striatum that initially released the larger amount of dopamine would be depleted to a greater extent than the contralateral side where now the postsynaptic dopamine content would be relatively higher. Again,

postsynaptic differences would be large, but now the animal would rotate contralateral to the direction of rotation elicited by the low doses of d-amphetamine. Thus at high doses of the drug, the selective release from one striatum would lead to the eventual depletion of dopamine on that side and the release of more transmitter from the contralateral side. If this is the case, then rats should not rotate to the same direction with low and high doses of d-amphetamine. Figure 2 indicates that the 20.0 mg/kg dose of amphetamine produced more frequent rotations to the opposite direction than the 1.25 mg/kg dose. This suggests that the NISTS contralateral to the one stimulated by 1.25 mg/kg was more active with the 20.0 mg/kg dose of amphetamine. When rats were tested twice, a week apart, with 1.0 mg/kg of d-amphetamine (Table 5), the direction of rotation remained unchanged for each rat and the magnitude of rotation was significantly correlated. After a dose of approximately 1.0 mg/kg of d-amphetamine, catecholamine levels are back to normal within 24 hours or less (Brodie et al., 1970; Leonard & Shallice, 1971), and even after higher doses, the drug or its metabolites are virtually eliminated in about 48 hours (Costa & Groppetti, 1970). In fact rats given 0.8 mg/kg every day for two weeks showed no change in catecholamine levels (Brodie et al., 1970). Thus testing the rats on Day 1 with 1.0 mg/kg of d-amphetamine, in the present investigation, would not be expected to influence the direction and magnitude of rotation induced by the drug on Day 8. In contrast to the consistent direction of rotation after the weekly administration of 1.0 mg/kg of d-amphetamine, rats did not necessarily

rotate to the same direction on Day 1 and Day 8 when they were tested with 1.0 and 20.0 mg/kg, respectively, of d-amphetamine (Table 8). Although all the rats tested on Day 8 did not have net rotations for the entire hour opposite to the direction of rotation observed on Day 1, every rat tested with 20.0 mg/kg did rotate initially, during the first few minutes of the test session, to the same direction as that observed on Day 1. However, during the last 30 minutes of the test session on Day 8, a preponderance of rotations, opposite to the initial direction of rotation, were recorded.

That amphetamine-induced rotation in normal rats is mediated via dopamine is supported by the AMPT, haloperidol, and DDC dose-response interactions (Tables 2, 3, & 4, respectively). Both AMPT, the inhibitor of tyrosine hydroxylase, and haloperidol, the dopaminergic blocker, significantly reduced the rotation elicited by the low doses (i.e. 1.0 & 2.5 mg/kg) of d-amphetamine. At the higher doses (greater than 5.0 mg/kg), neither AMPT nor haloperidol significantly reduced net rotations. However, with 25.0 mg/kg, the time course of amphetamine-induced rotation in rats pretreated with either AMPT or haloperidol, was markedly different from that induced by amphetamine alone (Figure 3). Both AMPT and haloperidol prevented the initial burst of rotation following the injection of amphetamine. In fact, the time courses shown in Figure 3 of the combined AMPT-amphetamine and haloperidol-amphetamine treatments bare a closer resemblance to the effects of lower doses of amphetamine. When rats were tested one week with 1.0 mg/kg of d-amphetamine and the following week with 1.0 mg/kg

of d-amphetamine, after prior treatment with 150.0 mg/kg of AMPT (Table 11), there was a marked inhibition of amphetamine-induced rotation on Day 8.

The results discussed thus far implicate catecholamines as the mediators of amphetamine-induced rotation in normal rats. Serotonin, on the other hand, does not appear to be involved in the rotational behavior of lesioned animals (Marsden & Guldberg, 1973), and in normal rats the cerebral content of the indolamine is unaffected by doses of d-amphetamine ranging from 1.0 to 30.0 mg/kg (Costa & Groppetti, 1970; Leonard & Shallice, 1971; Moore, Carr, & Dominic, 1970). The fact that DDC, an inhibitor of the copper containing enzyme--dopamine-beta-hydroxylase (Axelrod, 1972; Carlsson, Lindqvist, Fuxe, & Hokfelt, 1966; Collins, 1965; Goldstein, 1966; Lippmann & Lloyd, 1969; Maj, Grabowska, & Kwieck, 1970; Moore, 1969), did not inhibit the rotation induced by d-amphetamine (Table 4) confirms the idea that dopamine is the neurochemical substrate for drug-induced rotation in the normal rat. Ungerstedt (1971b) reported that inhibition of dopamine-beta-hydroxylase with FLA63 actually increased the rotation elicited by amphetamine (5.0 mg/kg) in rats with unilateral SN lesions. Similarly in Table 4, there is an overall though non-significant increase in rotation with DDC. Ungerstedt (1971b) proposed that changes in norepinephrine content possibly could modulate rotational behavior, and this suggestion is not entirely speculative in light of the more recent findings of a catecholamine sensitive adenylate cyclase in the rat brain (Clement-Cormier, Kebabian, Petzold, & Greengard, 1974; Kebabian, Petzold, & Greengard,

1972; Sholnick & Daly, 1974). This enzyme has been proposed to be the receptor for dopamine in the mammalian brain (Kebabian et al., 1972). In homogenates of rat caudate nuclei, dopamine was 7 times (4 μM vs 28 μM) more effective than norepinephrine in producing a half-maximal increase in adenyl cyclase activity. However, since various concentrations of dopamine plus norepinephrine did not increase enzyme activity above the maximum observed for either of the catecholamines alone, Kebabian et al. (1972) and Clement-Cormier et al. (1974) concluded that dopamine and norepinephrine stimulated the same receptor (i.e. adenyl cyclase); presumably dopamine had a greater intrinsic activity than norepinephrine. Thus if dopamine is the neurochemical mediator of rotation, pharmacological agents such as DDC, which reduce norepinephrine to about 40% of control values (Carlsson et al., 1966; Collins, 1965) without significantly affecting striatal dopamine content (Carlsson et al., 1966), would be expected to augment the rotation induced by the administration of d-amphetamine. It is possible also that other noradrenergic systems, extra-striatal in origin, may modulate amphetamine-induced rotation. Although the frontal cortex has been shown to influence drug-induced rotation (Glick & Greenstein, 1973), it is not known if these cortical influences are mediated via norepinephrine. The locus coeruleus appears to be the origin of noradrenergic terminals which ramify throughout the entire rat brain, especially the hippocampus and the cerebral and cerebellar cortices (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971a). Unilateral lesions of this nucleus cause an ipsilateral reduction of cortical norepinephrine and its

metabolite 3-methoxy-4-hydroxphenylglycol (MHPG) (Arbuthnott, Christie, Crow, Eccleston, & Walter, 1973; Korf, Aghajanian, & Roth, 1973). Such unilateral lesions, either ipsilateral or contralateral to the dominant direction of rotation in the normal rat, could be an effective means of investigating the postulated noradrenergic modulation of amphetamine-induced rotation. Although the true significance of the reported increase in rotation with FLA63 (Ungerstedt, 1971b) is questionable, since the investigator's data were not analyzed statistically, the evidence for dopaminergic-noradrenergic involvement at the same receptor suggests that these interactions, never the less, may be important for the modulation of rotational behavior.

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 right and left NISTS. Along with this explanation there is the tacit 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., 1973a; Ernst, 1965, 1967; Ungerstedt, 1971b), the rotation elicited by the dopaminergic agonist points to the existence of an intrinsic postsynaptic asymmetry in the NISTS of normal rats. Either there are more receptors in one striatum as compared to

the other, or there exists an unequal bilateral postsynaptic sensitivity to the action of apomorphine and presumably dopamine. The foregoing assumptions could explain the lack of a biphasic dose-response relationship for apomorphine-induced rotation (Figure 4). With small doses of apomorphine, differential stimulation of the receptors in the two striati 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 or even begin to decline with further increases in dose; perhaps it is relevant that in vitro studies have shown both apomorphine and dopamine, in relatively high concentrations (100 μM and greater) produce less than an optimal increase in the adenyl cyclase activity of caudate homogenates (Kebabian et al., 1972; Von Voigtlander, Boukma, & Johnson, 1973).

The transport of apomorphine into the brain must be rather fast, since maximum rotational behavior is observed within the first five minutes after the injection of a dose of 10.0 mg/kg (Figure 5). Similarly the drug is rapidly metabolized by a variety of pathways which include: 10-(mostly) and 11-O-glucuronidation (Kaul, Brochmann-Hanssen, & Way, 1961a, 1961b, 1961c); O-methylation with the formation of either apocodeine (10-OCH_3, mostly) or isoapocodeine (11-OCH_3) (Cannon, Smith, Modiri, Sood, Borgman, & Aleem, 1972; McKenzie & White, 1973; White & McKenzie, 1971); N-dealkylation (Smith & Sood, 1971); and auto-oxidation with the formation of unidentified decomposition products (Kaul et al., 1961b). As with the consistency data for amphetamine, apomorphine, injected on Day 1,

or its metabolites should not be present on Day 8 to influence the direction and magnitude of rotation observed on that day, after the administration of apomorphine (Table 6). In fact the magnitude of rotation is extremely consistent, despite some evidence which indicates that apomorphine can induce its own metabolism (Kaul & Conway, 1971); if this were an important factor, one week later, significantly less net rotations on Day 8 than on Day 1 might be expected. Although apomorphine is a substrate for catechol-O-methyltransferase (COMT), it is the drug itself and not the O-methylated metabolites which cause the stereotypic behaviors (Cannon et al., 1972; McKenzie & White, 1973; White & McKenzie, 1971) and presumably the rotation elicited in normal rats. Inhibition of COMT markedly enhanced the intensity and duration of stereotypy induced in the rat by apomorphine (0.5 - 20.0 mg/kg) administration (McKenzie & White, 1973; White & McKenzie, 1971), and apocodeine or isoapocodeine, in doses many times greater than apomorphine, did not initiate compulsive gnawing in mice or evoke emesis in pigeons (Cannon et al., 1972). Moreover, in the rat brain the lowest activity of COMT is found in the corpus striatum (Broch & Fonnum, 1972).

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 striati. Some have proposed that apomorphine's site of action is not entirely postsynaptic (Costall & Naylor, 1973; Goldstein et al., 1970). As mentioned previously, there is considerable evidence indicating

that the functional activity of the NISTS is regulated by a combination of feedback control mechanisms which include: an extra-striatal neuronal feedback loop; an intra-striatal receptor mediated control; and an intraneuronal neurochemical end-product inhibition. Through the action of one or all of these feedback control mechanisms, pharmacological agents which mimic the effect of dopamine, either by release of the transmitter or by direct stimulation of the receptors, can decrease the turnover of dopamine in the striatum. In addition, Goldstein et al. (1970) have demonstrated the *in vitro* and *in vivo* inhibition of tyrosine hydroxylase by apomorphine. However, if the mechanism of apomorphine-induced rotation were via differential dopamine metabolism in the two striati, then AMPT would be expected to decrease the rotation elicited by apomorphine. Since such a decrease did not occur, the proposed bilateral receptor asymmetry in the striati of normal rats is the most plausible explanation for the present data.

The importance of an intrinsic nigro-striatal postsynaptic asymmetry was most evident after unilateral caudate lesions. When apomorphine is administered to rats with unilateral lesions of the caudate nucleus, they will rotate toward the lesioned side (Andén, 1970). All rats tested with apomorphine in the present investigation also rotated ipsilateral to the lesion. However, the magnitude of postoperative rotation was considerably influenced by the direction of the animal's preoperative rotational preference; rats rotated more postoperatively if the lesion was made ipsilateral rather than contralateral to their preoperative direction of rotation (Table 15). In other

words, it mattered in which striatum each rat was lesioned. Ungerstedt (1971b) reported that some non-drugged unilaterally lesioned SN rats exhibit a slow spontaneous rotation contralateral to the lesioned side, one hour after the operation. This spontaneous contralateral rotation is in contrast to the ipsilateral direction of rotation observed in most animals after identical treatment. These paradoxical results could be explained by individual differences in the time course for the degeneration release of dopamine (Ungerstedt, 1971b). However in light of the present findings, it is possible also that some rats received the unilateral SN lesions contralateral to their normal pre-operative direction of rotation. It should no longer be assumed that a surgical insult to a structure in one hemisphere will have the same behavioral consequences as an identical perturbation in the contralateral hemisphere.

Thus far two mechanisms, one pre-and the other postsynaptic, have been postulated to account for drug-induced rotation in normal rats. The fact that rats tested with apomorphine one week and d-amphetamine the following week do not necessarily rotate in the same direction with each drug (Table 8), suggests that both pre-and postsynaptic nigro-striatal asymmetries exist, and that rotational behavior cannot be exlained adequately by a single mechanism. It may be postulated that the striatum which has a more sensitive release mechanism for dopamine also has less sensitive receptors. Since the direction of rotation induced by amphetamine is presumably the result of both pre-and postsynaptic asymmetries, whereas that of apomorphine is attributed only to the postsynaptic asymmetry, it would be surmised that rats which rotate in the same direction with each drug have pre-and postsynaptic striatal asymmetries which functionally act on the same side (i.e.

the dominant asymmetry is a postsynaptic receptor sensitivity). In animals where the direction of rotation is different for each drug , the two types of asymmetries would function opposite to each other (i.e. the presynaptic asymmetry is prepotent for amphetamine's action). Thus, such pre-and postsynaptic striatal asymmetries in the normal rat may affect behavior, and the direction of rotation in particular, in a way similar to that shown for lesioned animals. Following unilateral caudate lesions, rats rotate towards the lesioned side when either d-amphetamine or apomorphine is administered systemically, since there are fewer pre-and postsynaptic sites, ipsilateral to the lesion, at which amphetamine and apomorphine can act, respectively. However, after unilateral SN lesions and subsequent degeneration of the nigro-striatal pathway, the supersensitive receptors in the denervated striatum elicit rotation toward the unoperated side upon apomorphine administration, whereas amphetamine's release of dopamine from the intact striatum elicits rotation in a direction opposite to that induced by apomorphine (Ungerstedt, 1971b).

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, likewise would elicit rotational behavior in normal animals. In rats with unilateral lesions or inactivation (i.e. spreading depression) of the NISTS, L-DOPA has been reported to elicit rotation (Anden et al., 1966) or body torsion (Keller et al., 1973), respectively, contralateral to the normal side. The drug also evoked stereotypy

in normal rats (Butcher & Engel, 1969a), and dyskinesias in monkeys similar to the abnormal movements manifested clinically in parkinsonian patients undergoing L-DOPA therapy (Goldstein, Battista, Ohomoto, Anagnoste, &Fuxe, 1973; Mones, 1972). Generally, L-DOPA is administered, both clinically and experimentally, in conjunction with an inhibitor of the peripheral L-aromatic amino acid decarboxylase (i.e. DOPA decarboxylase). Thus the peripheral enzyme, including the DOPA-decarboxylase of the endothelial cells of the brain capillaries is inhibited (especially the enzyme of the striatum) (Bartholini & Pletscher, 1972). As a consequence, the unaltered amino acid now can penetrate into the brain where it is decarboxylated to form catecholamines (Bartholini & Pletscher, 1969; Bartholini & Pletscher, 1972; Bartholini, Constantinidis, Tissot, & Pletscher, 1971). Without the inhibition of DOPA-decarboxylase, marked peripheral autonomic signs (e.g. salivation, piloerection) are evident in rats treated with L-DOPA (Butcher & Engel, 1969a; 1969b). Among the various potent inhibitors of DOPA-decarboxylase, both Ro 4-4602 [N-(DL-seryl)-N'-(2,3,4-trihydroxybenzyl)hydrazine] and NSD 1015 (m-hydroxybenzylhydrazine) in high doses (i.e. about 0.16 mmol/kg or greater) penetrate the brain and inhibit cerebral DOPA-decarboxylase, whereas MK 486 does not (Bartholini & Pletscher, 1969; Porter, 1971). There is evidence which indicates that Ro 4-4602 also inhibits COMT (Bartholini, Blum, & Pletscher, 1969; Bartholini & Pletscher, 1969). 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.

The magnitude of rotation was small compared to that of d-amphetamine or apomorphine (Figure 6), and the direction of rotation was not consistently in the dominant direction during the hour test session (Figure 7). Although there is some behavioral evidence which indicates that L-DOPA may also have a dopaminergic receptor stimulating action (Creese & Iversen, 1973), neurophysiological data tends to refute the proposed postsynaptic effect (Bunney et al., 1973a).

Assuming that the rates of presynaptic uptake and decarboxylation of L-DOPA are identical in both striati, then bilateral differences in the normal physiological release of newly synthesized dopamine once again could explain pharmacologically induced rotation in the normal rat. Although presynaptic dopamine levels would be high after L-DOPA administration, release of the amine presumably would depend upon normal impulse activity of the NISTS. Since the physiological release of dopamine would not be expected to have an effect as great as the massive release produced by d-amphetamine or the direct pharmacological stimulation of the receptor (i.e. by apomorphine), L-DOPA-induced rotation could be expected to be small in magnitude. In agreement with the above proposal, neurophysiological studies have shown that doses of 0.10 and 0.25 mg/kg of apomorphine and d-amphetamine, respectively, markedly decreased the firing rates of nigral cells, whereas 25.0 mg/kg of L-DOPA, in conjunction with Ro 4-4602, was needed to produce a comparable effect (Bunney et al., 1973a).

Haloperidol-induced rotation

Haloperidol, the butyrophenone with a high dopaminergic to noradrenergic blocking ratio, has been reported to: produce catalepsy in normal rats, with doses of 0.5 and 2.0 mg/kg ((Costall & Olley, 1971; Naylor & Olley, 1972), arrest amphetamine-induced rotation in rats with unilateral lesions of the SN, at the dose of 1.0 mg/kg (Ungerstedt, 1971b), and elicit body torsion toward the intact side in unilaterally striatotomized rats, in doses of 1.0 (Anden & Bedard, 1971) and 2.0 mg/kg (Anden et al., 1966). Presumably, these results are due to haloperidol's blocking properties in the two striati. A bilateral block would produce catelepsy, 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 (Figure 8), the direction of which appeared to be relatively constant throughout the test session (Figure 9). It is possible that low doses of haloperidol create only a partial block of the striatal receptors. Now due to the bilateral differences in striatal receptor sensitivity, previously suggested, the partial block would be bilaterally asymmetrical. That is, the two striati 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 (Goodman & Gilman, 1970), it may be that rotation elicited by haloperidol is due to such a stimulant effect of the drug. Groves, Rebec, and Segal (1974) demonstrated that d-amphetamine (0.5 - 4.0 mg/kg) caused a marked

increase followed by a prolonged decrease in the firing rate of spontaneously active cells of the rat caudate nucleus. Haloperidol (2.0 mg/kg) also produced a transient increase, comparable to the amphetamine effect, followed by a return to the control levels of unit activity. It is possible that a dose of haloperidol of 0.25 mg/kg or less, might produce an effect even more similar to that produced by amphetamine.

Cholinergic influences on rotational behavior

As mentioned previously, there is considerable evidence which indicates that striatal cholinergic receptors are muscarinic (Keller et al., 1973; McLennan & York, 1967). More recently, in vivo studies have shown that the binding of ^{3}H-quinuclidinyl benzilate (QNB), a compound with a relatively high affinity for muscarinic receptors, was greatest in the rat striatum. Pretreating the animals with atropine largely prevented the accumulation of QNB (Yamamura, Kubar, & Snyder, 1974). In the present investigation, the anti-cholinergic drug of choice was scopolamine, since its central actions are about ten times that of atropine, while its peripherial effects are only twice as great (Parks, 1965). Doses of scopolamine, ranging from 1.0 to 200.0 mg/kg, induced significant rotation in normal rats. This finding is in contrast to the absence of any asymmetrical behavior in unilaterally striatotomized rats treated with comparable doses of scopolamine (Andén & Bedard, 1971). However, these investigators did not test for rotation in a rotometer; furthermore if rotational behavior is modulated by the frontal cortex (Glick & Greenstein,

1973), then removal of the striatum by suction (as done by Andén & Bedard, 1971) would include considerable ablation of the frontal cortex and would be expected to affect drug-induced rotation.

The transport of scopolamine into the brain must be rapid since rotational behavior is observed almost immediately after its administration (Figure 10). Assuming that the biotransformation of the drug is similar to that of atropine, then the anticholinergic is rapidly metabolized by a variety of pathways which include: deesterification (with subsequent excretion of tropic acid); mono- and dihydroxylation (in the m and p positions of the tropic acid moiety); mono- and di-O-glucuronide formation (in m and p positions); and unidentified products formed by modifications of the tropine base (Gaborel & Gosselin, 1958; Gosselin, Gaborel, Kalser, & Willis, 1955; Kalser, Willis, Gaborel, Gosselin, & Epes, 1957). Surely then, the anticholinergic administered on Day 1, or its metabolites, should not be present on Day 8 to influence scopolamine-induced rotation. As with amphetamine and apomorphine the direction of scopolamine-induced rotation was consistent from week to week (Table 7), and in addition, rats which rotated in one direction with d-amphetamine also turned to the same side with scopolamine (Table 9). The consistency of direction between amphetamine- and scopolamine-induced rotation in normal rats is in agreement with the results of the intracerebral drug-induced studies previously described. Both d-amphetamine and atropine produced contraversive turning after their unilateral infusion into the striatum (Costall et al., 1972; McKenzie

et al., 1972). 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.

It has been proposed that the dopaminergic and cholinergic systems are in reciprocal balance--the activity of one tends to counteract the activity of the other (Bartholini & Pletscher, 1972; Costall et al., 1972; Keller et al., 1973; Mennear, 1965). However, there is some evidence which indicates that anticholinergics may exert their effects via dopamine, per se. McKenzie et al.(1972) reported that atropine caused an increase in the resting release of basic catechols, including dopamine, from the prefused caudate nucleus of the cat. Whether this release is mediated via a cholinergic interneuron or is a direct presynaptic effect of the drug has not been ascertained. In a congerneric series of tropine related compounds, Horn, Coyle, and Snyder (1971) have shown noncompetitive reversible inhibition of dopamine uptake into synaptosomes prepared from the rat striatum. However, benztropine, an anticholinergic which did not elicit rotation after unilateral infusion into the rat striatum (McKenzie et al., 1972), was over 1600 times more potent than scopolamine in inhibiting the uptake of dopamine. Thus if scopolamine-induced rotation in normal rats were due solely to the "cocaine-like" action of the drug, it might be expected that a greater magnitude of rotation could be elicited by benztropine.

Although unilateral striatal infusions of arecoline have

been reported to elicit rotation (Costall et al., 1972), it appeared that pilocarpine, a cholinomimetic without the nicotinic actions of arecoline (Goodman & Gilman, 1970), would be better suited as a test drug in the present investigation, since evidence previously discussed indicated that the cholinergic receptors in the caudate were predominantly muscarinic in nature. At all doses tested, pilocarpine did not elicit significant rotation in normal rats, and it significantly reduced the magnitude of rotation induced by d-amphetamine (Table 13). It has been reported that 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 & 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. Thus pilocarpine which might simulate an increase in ACh levels, would be expected not to induce rotation and to antagonize rotation induced by d-amphetamine. Scopolamine, on the other hand, conceivably could counteract the behavioral effect of the amphetamine-induced rise in ACh, and either potentiate or not affect the drug-elicited rotation, depending upon whether or not the rotation with amphetamine alone was already maximal (Table 12).

Neurochemical correlates of rotation

The values obtained for tel-diencephalic and striatal d-amphetamine, in the present investigation, are not directly

comparable with cerebral amphetamine levels previously reported in the literature, since other investigators used animals differing from the present study in species (Axelrod, 1970), strain (Fuller, Snoddy, & Molloy, 1973), or sex (Brodie et al., 1970; Maickel, Cox, Miller, Segal, & Russell, 1969; Sulser et al., 1967), determined whole brain amphetamine levels at times other than one-half hour, used selected doses of amphetamine without attempting to determine a dose-response relationship, and did not contain the animals in a rotometer before decapitation. It is evident from Figure 13 that the tel-diencephalic and striati dose-response relationships are nearly identical. The amphetamine content in both neuroanatomical structures can be described adequately by the same linear equation: $\mu g/g = 1.76(\text{dose}) - 1.16$. Thus it appears that in the rat brain, there is no unequal regional distribution between the structures examined, and in addition there are no left-right differences for each individual structure (Table 16).

One major criticism to the previously offered explanations of amphetamine-induced rotation in normal rats is the possibility that the unequal release of dopamine from the striati is due to the unequal distribution of the drug to the two sides of the brain. Thus amphetamine would release more dopamine from one striatum simply because there was more drug present on that side as compared to the other. If the previous statement were true, then there would have to exist a vascular asymmetry between the two striati of each rat, since as previously discussed, the direction and magnitude of rotation

from week to week was extremely stable. Glick et al. (1974) reported that there were no bilateral differences in the striatal ACh content after 20.0 mg/kg of d-amphetamine, even though there was a significant drug-induced increase in ACh. However, bilateral differences in striatal dopamine content were found. If amphetamine were unequally distributed to the striati, it would be highly improbable that bilateral differences in dopamine and ACh content would not be affected similarly. The present findings directly establish that there are no bilateral asymmetries in the distribution of d-amphetamine, since there were no significant differences between the amphetamine levels ipsilateral and contralateral to the direction of rotation (Table 16). At each dose, striatal amphetamine was not found to be consistently higher either ipsilaterally or contralaterally to the direction of rotation. Thus, in view of the present neurochemical findings, the argument that amphetamine-induced rotation is a drug distribution phenomenon, resulting from differences in striatal vascularization, is no longer tenable.

After the administration of d-amphetamine, catecholamine brain levels in the rat have been reported to decrease (Besson, Cheramy & Glowinski, 1971; Brodie et al., 1970; Moore & Lariviere, 1963), increase (Costa, Groppetti, & Naimzada, 1972; Smith, 1965), or remain unchanged (Costa & Groppetti, 1970). The effect of amphetamine on catecholamine content appears to be dose related. There is general agreement that at high doses of d-amphetamine (i.e. greater than 5.0 mg/kg), brain catecholamine levels in rats are significantly reduced about

40% (after 3 hrs with 10 mg/kg--Brodie et al., 1970; after 2 hrs with 10.0 mg/kg--Leonard & Shallice, 1971; after 30 min with 20.0 mg/kg--Glick et al., 1974). However, the apparent conflicting reports of catecholamine depletion at low doses of d-amphetamine, may be due to a biphasic action of the drug on catecholamine synthesis. Tyrosine hydroxylase activity was inhibited in striatal homogenates (Harris & Baldessarini, 1973) and slices (Besson et al., 1971) 30 and 90 minutes, respectively, after the administration of 5.0 mg/kg of d-amphetamine. The decline in enzyme activity was evident only after the homogenates had been incubated 15-30 minutes (Harris & Baldessarini, 1970). It was also reported that d-amphetamine in concentrations of 10^{-4} and 10^{-7} M noncompetitively inhibited tyrosine hydroxylase in vitro (Besson et al., 1971). Since AMPT decreased the brain concentration of p-hydroxyamphetamine, it is possible that amphetamine may be a substrate for the enzyme (Costa & Groppetti, 1970). Although d-amphetamine inhibits the reuptake of catecholamines, apparently the drug is not a substrate for this transport system (Iversen, 1971). Thus it is difficult to conceive of how amphetamine itself could inhibit an intraneuronal enzyme. Since these investigators did not measure endogenous dopamine, it is entirely possible that amphetamine initially stimulated tyrosine hydroxylase by reducing impulse flow in the NISTS. The proposed increase in enzyme activity would raise striatal dopamine levels to the point where end-product inhibition now would reduce the activity of tyrosine hydroxylase. Measuring enzyme activity at this time would indicate that there had been

a decrease. Besson et al. (1971) reported that even at 1.0 mg/kg of d-amphetamine the formation of ^{3}H-dopamine was reduced to about 60% of control values, in striatal slices incubated with ^{3}H-tyrosine. In contrast, other investigators have demonstrated an increase in the turnover of striatal dopamine following 1.0 mg/kg (i.p.) (Costa & Groppetti, 1970) or 0.3 mg/kg (i.v.) (Costa et al., 1972) of d-amphetamine. Aside from the methodological differences among the studies, measurements of dopamine turnover made within 45 minutes after the administration of d-amphetamine showed an increase (Costa & Groppetti, 1970; Costa et al., 1972), whereas a decrease was evident 90 minutes after the drug (Besson et al., 1971). These results are consistent with the proposed biphasic action of amphetamine. Neurochemical measures on animals killed shortly after the administration of low doses of d-amphetamine would show an increase in turnover, due to the neuronal feedback stimulation of tyrosine hydroxylase, whereas a decrease would be observed in animals killed later due to the accumulation of dopamine and subsequent end-product inhibition. As previously discussed, the firing rates of spontaneously active cells of the caudate nucleus are first increased for about 30 minutes following the administration of d-amphetamine, then depressed below the control rates of firing (Groves et al., 1974). Perhaps these biphasic neurophysiological events are correlative of the proposed stimulatory and subsequent inhibitory actions of d-amphetamine on tyrosine hydroxylase

In the present investigation, 2.0 - 10.0 mg/kg of d-amphetamine significantly increased brain catecholamine levels,

whereas the dose of 20.0 mg/kg resulted in a significant depletion. Leonard and Shallice (1971) reported a significant increase in whole brain catecholamines only after the administration of 1.0 mg/kg of d-amphetamine; the dose of 5.0 mg/kg resulted in depletion which became significant at 10.0 mg/kg. Aside from the fact that Leonard and Shallice (1971) assayed for both norepinephrine and dopamine in whole rat brain, whereas the present determination of each amine was made regionally, the apparent discrepancies between the results of both studies may be resolved by considering the time at which the rats were killed after drug administration. Leonard and Shallice (1971) killed rats 2 hours after injection; animals were decapitated after 30 minutes in the present study. At the latter time, stimulation of tyrosine hydroxylase by amphetamine first would increase catecholamine levels. Later on in time, as the enzyme was inhibited by excess products, synthesis of the amines would not be able to keep pace with their release and catecholamine levels would decline.

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 confirm the hypothesis that amphetamine-elicited rotational behavior in normal rats is due to a bilateral imbalance of striatal dopamine. As previously reviewed, systemic administration of amphetamine to rats with unilateral NISTS lesions results in rotation towards the operated side and

unilateral intrastriatal infusions of dopamine produce contraversive turning: the animal rotates to the side containing the lowest dopamine content. Similarly, the present findings demonstrate that at 20.0 mg/kg of d-amphetamine normal rats also rotate to the side containing the least dopamine (Table 16). However, at the 2.0 mg/kg dose normal rats rotate to the side where dopamine content was higher. The apparent paradox is resolved after further consideration of amphetamine's action in the striatum. It is proposed that as amphetamine begins to release dopamine from both striati, neuronal feedback would stimulate tyrosine hydroxylase to synthesize more of the amine. However, in accordance with the mechanism of amphetamine-induced rotation, the drug would release more dopamine from the contralateral striatum than the striatum ipsilateral to the direction of rotation. Postsynaptically, the released amine would be more susceptible to transport out of the caudate, and catabolic processes (Axelrod, 1970). On the other hand, the striatum ipsilateral to the direction of rotation would contain more newly synthesized presynaptic dopamine, which apparently is protected from enzymatic degradation by MAO and COMT (Besson et al., 1971). Thus as expected, the animal would rotate to the side where the postsynaptic content of dopamine was lower. However, measuring dopamine at this dose (i.e. 2.0 mg/kg) would indicate that the ipsilateral striatum contained more of the amine than its contralateral counterpart. With larger doses of d-amphetamine, the contralateral striatum would become rapidly depleted and the other striatum would begin to release more of the newly syn-

thesized amine; the animal would then reverse rotational direction. Neurochemical analysis at this dose (i.e. 20.0 mg/kg) would indicate higher dopamine levels in the striatum opposite to the direction of rotation. Although the preceding explanation is tenable, it should be regarded as highly speculative at this time.

Rotation in non-drugged rats is rather minimal (i.e. 1 or 2 rotations per 30 minutes) and is not distinguishable from random activity. Thus the validity of comparisons between dopamine levels ipsilateral and contralateral to the direction of rotation, for the saline control animals, is questionable. It is now established that the bilateral imbalance of striatal dopamine is the neurochemical substrate for the rotation elicited by amphetamine in normal rats. Although it is not clear, from the present investigation, whether amphetamine induced the imbalance or exacerbated a neurochemical asymmetry normally present in rats., Zimmerberg, Glick, and Jerussi (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.

Rotational behavior: some theoretical considerations

Rats with unilateral lesions of the NISTS will rotate also without drugs, when subjected to so-called stressful stimuli (e.g. tail pinching, loud noises, etc.) (Ungerstedt, 1971b), and noxious stimuli are reported to augment drug-induced rotation (McKenzie et al., 1972). In the present investigation, it was observed that with low doses of the drugs tested, rats did not rotate outside the rotometer, and after being tested

in the rotometer the rats appeared very aggressive (e.g. indiscriminate biting of objects, squealing, retraction of ears) upon their removal from the apparatus. This behavior was more than just stereotypy induced by the test drug, since biting was directed to the object and was observed after administration of scopolamine or doses of d-amphetamine which have not been reported to elicit stereotypic behaviors in rats. It appeared that the stressful environment of the test apparatus augmented or modified the behavioral effect of the drug, and thus rotation could be elicited in the normal rat. If the conclusions drawn from these observations are valid, what then is the normal physiological relationship between stress or heightened arousal and rotational behavior, and what is its possible adaptive significance?

A "caudate-loop" has been described within which the caudate nucleus receives inputs from the CM nucleus of the thalamus and conveys modified signals to the cortex via the VA thalamic nucleus (Buchwald, Heuser, Wyers, & Lauprecht, 1961a; Buchwald, Wyers, Okuma, & Heuser, 1961c; Heuser, Buchwald, & Wyers, 1961). Single shock stimuli delivered to the head of the caudate nucleus of the cat produced a short latency evoked response followed by a train of high voltage activity (about the frequency of alpha waves), termed the "caudate spindle", in the ipsilateral (mostly) and contralateral anterior sigmoid gyrus and also in the remaining neocortex. Apparently this negative feedback system functioned antagonistically to the ascending reticular activating system, since high frequency (300 pps) electrical stimulation of the midbrain reticular

formation could inhibit caudate spindles (Buchwald et al., 1961a). However similar stimulation of the caudate nucleus itself could also inhibit the spindling activity in the cortex. The bar-pressing of cats in an operant situation ceased concurrent with bilateral low frequency (0.2 - 5 pps) stimulation of the caudate, and control rates of responding could be quickly reinstated following stimulation with frequencies sufficiently high to desynchronize the caudate spindles (Buchwald, Wyers, Lauprecht, & Heuser, 1961b). When bar-pressing had ceased, the animals appeared inattentive to the operant task, whereas high frequency stimulation produced behavioral signs of arousal and alertness. Thus it appeared that low frequency stimulation of the caudate did not directly impair motor performance, but rather disrupted arousal mechanisms necessary for attending to the task at hand. Jung and Hassler (1960) also implicated the striatum as a key component in arousal and attentive processes, and in reference to striatal function concluded: "Inhibition of cortical voluntary motor activity seems to be an essential condition for the individual to be able to concentrate temporarily on restricted sensory perceptions of specific motor performances (p. 876)."

More recently, Marshall, Turner, and Teitelbaum (1971) demonstrated a phenomenon called "sensory neglect" resulting from lesions of the lateral hypothalamus, which in all probability interrupted the more rostral extent of the nigro-striatal bundle. Rats with unilateral lateral hypothalamic lesions were inattentive to a variety of stimuli (e.g. food, whisker touch, etc.) presented and demonstrated a markedly impaired

orienting reflex to the side contralateral to the lesion. Again the deficit did not appear to be primarily motor in origin, for it was evident that the lesioned animals were capable of turning both head and body in the contralateral direction. Kirby (1973) reported that rats with bilateral lesions of the caudate nuclei defecated more, were more active, and in general were more reactive to stress than rats with control lesions. In addition, every type of dyskinesia previously reviewed, resulting from lesions of the basal ganglia, disappears when the patient is sleeping, and is exacerbated during emotional excitement (Jung & Hassler, 1960). Thus from the preceding discussion it is apparent that the striatum and undoubtedly the NISTS is more than a mere component of a motor system but is intimately associated with mechanisms of attention and arousal.

It appears that the caudate nucleus and the NISTS are not involved only with mechanisms of arousal, but their integrity is necessary for the normal expression of spatial behavior in rats. Potegal (1969) reported that bilateral lesions of the caudate nucleus, which although did not produce a learning deficit, significantly impaired the spatial orientation of rats in a maze. Zimmerberg et al., (1974) demonstrated that rats with unilateral lesions of the caudate nucleus and tested in a T-maze had post-operative side preferences ipsilateral to the lesion. Side preferences in normal rats were in the same direction as the rotation induced by d-amphetamine, and dopamine content in the striatum contralateral to the spatial preference was significantly higher than levels of the

amine on the ipsilateral side. However, no such correlation was found for telecephalic norepinepherine. These investigators suggest that drug-induced rotation is a stereotyped form of spatial behavior. This suggestion is further supported by work with scopolamine which elicits rotation and also affects spatial behavior. When rats are given two trials in a T-maze, the arm of choice in the second trial is usually opposite to that of the first (Dember & Fowler, 1958). This "spontaneous alternation" can be disrupted by scopolamine (Egger, Livesey, & Dawson, 1973). It would be interesting to see if the direction of the disruption of spontaneous alternation was the same as that of scopolamine-induced rotation.

Rotational behavior, in general, appears to be a feature of many an animal's motor repertoire. Rotatory movements about the longitudinal axis, which supposedly are indicative of emotional release, have been described during fighting and sexual play in cats (Jung & Hassler, 1960). In dogs, apomorphine induced circular running, the direction of which was constant for each animal (Nymark, 1972). Similarly, it has been observed that emotionally excited dogs will circle in a direction particular for that animal (Glick, unpublished observations). The "superstitious" turning and twisting of pigeons receiving noncontingent reinforcement in an operant situation (Skinner, 1948) indicated that these behaviors must have occurred with a greater incidence than other motor patterns. Anecdotally, the hawk circling a field in search of prey or humans pacing the floor during emotional unrest are stereotyped rotatory patterns of behavior. Thus it appears

that stereotyped rotational movements have a wide distribution among organisms and are particularly evident during states of high arousal when they are seemingly "released".

Thus far it has been discussed how the caudate and the NISTS seem to be involved in arousal and spatialness; now remains the task to explain how these two phenomena are related to drug-induced rotation. It is proposed that spatial behavior has evolved from a primitive bilaterally asymmetrical arousal function which initially consisted of the taxis (i.e. the reflexive movement directed toward or away from a particular stimulus) but in mammalia, for example, has evolved to the orientation reaction or orienting reflex. The automatic or reflexive nature of the taxis as well as the apparent deliberateness of the orientation reaction seem to have the same adaptive value--to prepare the organism to deal with novel or significant stimuli (Lynn, 1966). Stimuli impinging on the physiological receptors subsequently would be processed and evaluated by the cerebral cortex, and depending upon the relevance of the stimulus to the organism turning to the source of stimulation would be initiated. However, does the organism turn and attend or attend and turn? Perhaps these so-called motor and associative processes are inseparable, and the motor components involved in the act of turning actually facilitate attentive processes. Now consider an organism with arousal or arousal-modulating systems (i.e. possibly the NISTS) which have evolved to be functionally asymmetrical; that is one system has a lower threshold for arousal than its contralateral counterpart. Thus attending and turning (or

turning and attending?) to the source of stimulation on one side, would be accomplished with greater facility than attending and turning to stimuli on the opposite side. Since stimuli on one side could be more easily attended to, the organism would develop a spatial or side preference in that direction. The intimate neuronal connections between the striatum and thalamus make it anatomically possible for sensory stimuli to bypass the discriminative function of the cortex and influence the NISTS directly. Thus intense stimuli, such as noxious stimuli (e.g. tail pinching, loud noises, etc.) or pharmacological stimulation of the receptor (e.g. by amphetamine), would overload the system, bypass or short circuit cortical control, and reflexively turn the animal in the preferred direction.

Unlike the taxis, the orientation reaction quickly habituates, the rate of which appears to directly correlate with the development of the cerebral cortex (Lynn, 1966). It is interesting to note that rats with cortical lesions (Glick & Greenstein, 1973) exhibit much more rotational behavior after d-amphetamine than normal intact rats. Thus the exaggerated orientation reaction (i.e. rotation) expressed after the administration of amphetamine is still inhibited, to a certain degree, by the intact cortex. Inhibitory control can be released further by lowering the norepinephrine content of the cortex, as previously discussed

Since the drugs which induced rotation, in the present investigation, produced asymmetrical behavioral effects, and their distribution to the two sides of the brain were not

significantly different, it is evident from this and the neurochemical data that the rat brain (i.e. the NISTS) itself must posses a functional bilateral asymmetry. In man, such a hemispheric asymmetry (i.e. cerebral dominance) is behaviorally manifested as handedness. As far back as 1930 "handedness" or paw preference was investigated in the albino rat (Tsai & Maurer, 1930), and as in the present study with drug-induced rotation, paw preference appeared to be a stable phenomenon which remained unchanged for the life of the animal (Peterson, 1934). However, paw preferences in rats can be changed by surgical procedures and forced practice of the non-preferred limb (Peterson, 1934; Peterson & Barnett, 1961; Peterson & Devine, 1963). Small lesions (about 1 mm^3) of the frontal cortex, contralateral either to the preferred forepaw or the paw receiving forced practice, could initiate reaching for food with the opposite paw (Peterson & Barnett, 1961; Peterson & Devine, 1963). Although these studies implicate the cortex as the neuroanatomical substrate for handedness, the rat unlike man has a poorly developed cortico-spinal tract (i.e. many unmylenated axons; most fibers do not descend below the level of the medulla; no homolateral tract exists) which in all probability is only of secondary importance (King, 1910; Ranson, 1913). Thus it seems that most, if not all, motor functions in the rat are extrapyramidal. It is interesting that lesions which cause a transfer of handedness included an area of the frontal cortex which was reported (Glick & Greenstein, 1973) to produce rotation in amphetamine treated rats and to modulate striatal activity. The locus of the

effective lesions involved the deeper layers of the cortex (Peterson & Barnett, 1961; Peterson & Devine, 1963) and some of them appeared to have encroached upon subcortical structures (e.g. the caudate nucleus) (Peterson, 1934). Unilateral lesions of the caudate nucleus contralateral to the preferred limb have been shown to prevent bar-pressing with that paw, in an operant test chamber, while performance with the ipsilateral paw remained unaffected. However, when the contralateral cortex was ablated, three of the four rats tested continued to respond with the preferred paw, and the other rat was easily retrained to press as before (Hansing, Schwartzbaum, & Thompson, 1968). Similarly in this laboratory, after unilateral caudate lesions contralateral to the preferred paw, rats were made to reach with the non-preferred paw in order to obtain food (unpublished data). Thus it seems, as with spatial behavior, the normal expression of handedness in the rat also is dependent upon an intact striatum.

Again it appears that handedness, as with spatialness, could have evolved from the same primitive bilaterally asymmetrical arousal function (i.e. the NISTS), as previously proposed. As the organism preferrentially attended and turned to stimuli on one side (i.e. developed a spatial preference), it would tend to operate upon or manipulate these stimuli more frequently than similar stimuli on the contralateral side. Thus a spatial preference for one side would engender a greater motor proficiency ipsilaterally, and a motor preference or handedness would now emerge.

If spatial preference, handedness, and drug-induced rotation

in normal rats are expressions of the same neuroanatomical substrate (i.e. the NISTS), then these phenomena should be correlated with respect to the side, paw, and direction of the preference. Glick and Jerussi (1974) have demonstrated that spatial preferences, in an operant situation, were most stable if they were linked to a definite paw preference and could be enhanced by d-amphetamine if both lever and paw preference were to the same side. Side preferences have been positively correlated with the direction of amphetamine-induced rotation in normal rats (Glick & Jerussi, 1974; Zimmerberg et al., 1974) and a similar relationship for rotation and paw preference is now being investigated.

SUMMARY

Normal unoperated rats were tested for rotation in a spherical rotometer and dose-response relationships were generated using d-amphetamine, apomorphine, L-DOPA, haloperidol, and scopolamine. Since d-amphetamine-induced rotation was antagonized by alpha-methyl-p-tyrosine and haloperidol but not by diethyldithiocarbamate, it was concluded that the mechanism of amphetamine-induced rotation in normal rats was possibly the same as that proposed for rotation in rats with unilateral lesions of the nigro-striatal system (i.e. a differential release of dopamine in the two striati). This differential amphetamine-induced release of dopamine is presumably part of the rat's normal physiological make-up, since the direction and magnitude of rotation for each rat was consistant from week to week when tested with 1.0 mg/kg of d-amphetamine. However, since the directions of rotation induced by the 1.0 and 20.0 mg/kg doses of d-amphetamine were not necessarily the same, it was suggested that the 20.0 mg/kg dose of d-amphetamine first depleted the striatum which initially released more dopamine and then caused the animal to rotate in the opposite direction by now releasing more dopamine from the contralateral striatum. The possibility of noradrenergic systems which modulate rotational behavior was discussed.

The rotation elicited by apomorphine was unaffected by alpha-methyl-p-tyrosine, and like amphetamine, the direction and magnitude of rotation were consistent from week to week. Since apomorphine and amphetamine did not necessarily elicit rotation in the same direction, it was postulated that , in addition to a presynaptic asymmetry, there also existed a postsynaptic receptor asymmetry.

The importance of a nigro-striatal asymmetry was most evident after unilateral lesions of the caudate nucleus; rats rotated more post-operatively with apomorphine if the lesion was made ipsilateral rather than contralateral to the animal's pre-operative preference.

Haloperidol elicited significant rotation only at the low dose of 0.125 mg/kg. It was suggested that perhaps haloperidol had a stimulant effect which preceded its post-synaptic blocking action.

Dopaminergic-cholinergic interactions which influenced rotational behavior were evident. Pilocarpine antagonized amphetamine-induced rotation, whereas scopolamine did not antagonize but in fact elicited rotation in the same direction as that induced by amphetamine.

Bilateral regional neurochemistry was performed on rats injected with various doses of d-amphetamine and tested for rotation. The amphetamine content, after 30 minutes, in both tel-diencephalic halves and striati was linearly related to the dose of d-amphetamine sulphate administered. Doses of 2.0 and 5.0 mg/kg significantly raised tel-diencephalic norepinephrine and striatal dopamine levels. On the other

hand, catecholamine levels were significantly lowered by 20.0 mg/kg of d-amphetamine. The only bilateral neurochemical measure of significance was the difference in striatal dopamine content ipsilateral and contralateral to the direction of rotation induced by d-amphetamine. It was established that amphetamine-induced rotation was not due to an unequal distribution of the drug in the two striati. With the 2.0 mg/kg dose of amphetamine, dopamine content was higher in the striatum ipsilateral to the direction of rotation, whereas at 20.0 mg/kg the contralateral striatum contained significantly more dopamine. This apparent discrepancy was resolved by proposing a mechanism whereby the postsynaptic content of dopamine, at 2.0 and 20.0 mg/kg, would always be higher in the striatum contralateral to the direction of rotation.

The theoretical implications of the results were discussed in relation to arousal, spatial behavior, and handedness, and how these functions could have evolved from the same bilateral striatal asymmetry.

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